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(57) Abstract

Nucleic acid molecules are described encoding a starch granule-bound protein from maize as well as methods and recombinant DNA molecules for the production of transgenic plant cells and plants synthesizing a modified starch. Moreover, the plant cells and plants resulting from those methods as well as the starch obtainable therefrom are described.

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Novel nucleic acid molecules from maize and their use for the production of modified starch

The present invention relates to nucleic acid molecules encoding a starch granule-bound protein from maize as well as to methods and recombinant DNA molecules for the production of transgenic plant cells and plants synthesizing modified starch. The invention also relates to the transgenic plant cells and plants resulting from these methods and to the starch obtainable from the transgenic plant cells and plants.

The polysaccharide starch, which constitutes one of the most important storage substances in plants, is not only used in the area of foodstuffs but also plays a significant role as a regenerative material in the manufacturing of industrial products. In order to enable the use of this raw material in as many areas as possible, it is necessary to obtain a large variety of substances as well as to adapt these substances to the varying demands of the processing industry.
Although starch consists of a chemically homogeneous basic component, namely glucose, it does not constitute a homogeneous raw material. It is rather a complex mixture of various types of molecules which differ from each other in their degree of polymerization and in the degree of branching of the glucose chains. One differentiates particularly between amylose-starch,

a basically non-branched polymer made up of α-1,4-glycosidically branched glucose molecules, and amylopectin-starch which in turn is a mixture of more or less heavily branched glucose chains. The branching results from the occurrence of α-1,6-glycosidic interlinkings.
The molecular structure of starch which is mainly determined by its degree of branching, the amylose/amylopectin ration, the average chain-length and the occurrence of phosphate groups is significant for important functional properties of starch or, respectively, its watery solutions. Important functional properties are for example solubility of the starch, tendency to retrogradation, capability of film formation, viscosity, pastification properties, i.e. binding and gluing properties, as well as cold resistance. The starch granule size may also be significant for the various uses. The production of starch with a high amylose content is particularly significant. Furthermore, modified starch contained in plant cells may, under certain conditions, favorably alter the behavior of the plant cell. For example, it would be possible to decrease the starch degradation during the storage of the starch-containing organs such as seeds and tubers prior to their further processing by, for example, starch extraction. Moreover, there is some interest in producing modified starches which would render plant cells and plant organs containing this starch more suitable for further processing, such as for the production of popcorn or corn flakes from maize or of French fries, crisps or potato powder from potatoes. There is a particular interest in improving the starches in such a way, that they show a reduced "cold sweetening", i.e. a decreased release of reduced sugars (especially glucose) during long-term storage at low temperatures.
Starch which can be isolated from plants is often adapted to certain industrial purposes by means of chemical modifications which are usually time-consuming and expensive. Therefore it is

desirable to find possibilities to produce plants synthesizing a starch the properties of which already meet the demands of the processing industry.

Conventional methods for producing such plants are classical breeding methods and the production of mutants. Thus, for example, a mutant was produced from maize synthesizing starch with an altered viscosity (US patent specification 5,331,108) and a maize variety (waxy maize) was established by means of breeding the starch of which consists of almost 100% amylopectin (Akasuka and Nelson, J. Biol. Chem. 241 (1966), 2280-2285). Furthermore, mutants of maize and pea have been described which synthesize starches with a high amylose content (70% in maize or up to 50% in pea). These mutants have so far not been characterized on the molecular level and therefore do not allow for the production of corresponding mutants in other starch-storing plants.

Alternatively, plants synthesizing starch with altered properties may be produced by means of recombinant DNA techniques. In various cases, for example, the recombinant modification of potato plants aiming at altering the starch synthesized in these plants has been described (e.g. WO 92/11376; WO 92/14827). However, in order to make use of recombinant DNA techniques, DNA sequences are required the gene products of which influence starch synthesis, starch modification or starch degradation.

Therefore, the problem underlying the present invention is to provide nucleic acid molecules and methods which allow for the alteration of plants in such a way, that they synthesize a starch which differs from starch naturally synthesized in plants with respect to its physical and/or chemical properties and is therefore more suitable for general and/or particular uses.

This problem is solved by the provision of the embodiments described in the claims.

Therefore, the present invention relates to nucleic acid molecules encoding a protein comprising the amino acid sequence indicated in Seq ID No. 6 or in Seq ID No. 8. Such proteins are present in the plastids of plant cells, bound to starch granules as well as in free, i.e. soluble form.

The present invention further relates to nucleic acid molecules comprising a sequence with the nucleotide sequence indicated in Seq ID No. 5 or in Seq ID No. 7, particularly the coding region indicated in Seq ID No. 5 or in Seq ID No. 7.

Nucleic acid molecules encoding a protein from maize, which in the plastids of the cells is partly granule-bound, and hybridizing to the above-mentioned nucleic acid molecules of the invention or their complementary strand are also the subject matter of the present invention. In this context the term "hybridization" signifies hybridization under conventional hybridizing conditions, preferably under stringent conditions as described for example in Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).
More preferably hybridization occurs under the following conditions:

Hybridization buffer: 2 x SSC; 10 x Denhard's solution (Fikoll 400 + PEG + BSA; ratio 1:1:1); 0.1% SDS; 5 mM EDTA; 50 mM Na_2HPO_4; 250 µg/ml herring sperm DNA; 50µg/ml tRNA; or
0.25 M sodiumphosphate buffer pH 7.2
1 mM EDTA
7% SDS

Hybridization temperature T = 65 + 68°C

Washing buffer: 0.2 x SSC; 0.1% SDS

Washing temperature T = 40 to 68°C.

Nucleic acid molecules hybridizing to the molecules according to the invention may be isolated e.g. from genomic or from cDNA libraries produced from maize cells or tissue.

The identification and isolation of such nucleic acid molecules may take place by using the molecules according to the invention or parts of these molecules or, as the case may be, the reverse complementary strands of these molecules, e.g. by hybridization according to standard methods (see e.g. Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

As a probe for hybridization e.g. nucleic acid molecules may be used which exactly or basically contain the nucleotide sequence indicated under Seq ID No. 5 or under Seq ID No. 7 or parts thereof. The DNA fragments used as hybridization probe may also be synthetic DNA fragments which were produced by means of the conventional DNA synthesizing methods and the sequence of which is basically identical with that of a nucleic acid molecule of the invention. After identifying and isolating genes hybridizing to the nucleic acid sequences according to the invention, the sequence has to be determined and the properties of the proteins encoded by this sequence have to be analyzed.

Such hybridizing nucleic acid molecules also encompass fragments, derivatives and allelic variants of the above-mentioned nucleic acid molecules, which encode the above-mentioned protein. In this context fragments are described as parts of the nucleic acid molecules which are long enough in order to encode the above-described protein. The term derivative means that the sequences of these molecules differ from the sequences of the above-mentioned nucleic acid molecules at one or more positions and exhibit a high degree of

homology to the sequences of these molecules. Homology means a sequence identity of at least 40%, in particular an identity of at least 60%, preferably of more than 80% and still more preferably a sequence identity of more than 90% and particularly preferred of more than 95%. The deviations occurring when comparing with the above-described nucleic acid molecules might have been caused by addition, deletion, substitution, insertion or recombination.
Moreover, homology means that functional and/or structural equivalence exists between the respective nucleic acid molecules or the proteins they encode. The nucleic acid molecules, which are homologous to the above-described nucleic acid molecules and represent derivatives of these molecules, are generally variations of these nucleic acid molecules, that constitute modifications which exert the same biological function. These variations may be naturally occurring variations or mutations, whereby these mutations may have occurred naturally or they may have been introduced deliberately. Moreover the variations may be synthetically produced sequences.
The allelic variants may be naturally occurring as well as synthetically produced variants or variants produced by recombinant DNA techniques.
The proteins encoded by the various variants of the nucleic acid molecules according to the invention exhibit certain common characteristics. Enzyme activity, molecular weight, immunologic reactivity, conformation etc. may belong to these characteristics as well as physical properties such as the mobility in gel electrophoresis, chromatographic characteristics, sedimentation coefficients, solubility, spectroscopic properties, stability, pH-optimum, temperature-optimum etc.

Furthermore, the present invention relates to nucleic acid molecules the sequences of which, compared to the sequences of the above-mentioned molecules, are degenerated due to the genetic code and which encode a protein which is present in the plastids of plant cells partly in granule-bound and partly in free form, i.e. in a soluble form.

The nucleic acid molecules of the invention can, for example, be isolated from natural sources, produced by methods of genetic engineering, e.g. by PCR, or produced by means of synthesis methods known to the skilled person.

The nucleic acid molecules of the invention may be DNA molecules, such as cDNA or genomic DNA, as well as RNA molecules.

Furthermore, the invention relates to vectors, especially plasmids, cosmids, viruses, bacteriophages and other vectors common in genetic engineering, which contain the above-mentioned nucleic acid molecules of the invention.

In a preferred embodiment the nucleic acid molecules contained in the vectors are linked to regulatory elements that ensure the transcription and synthesis of a translatable RNA in prokaryotic and eukaryotic cells.

In a further embodiment the invention relates to host cells, in particular prokaryotic or eukaryotic cells, which have been transformed and/or recombinantly manipulated by an above-mentioned nucleic acid molecule of the invention or by a vector of the invention, as well as cells which are derived from such cells and which contain a nucleic acid molecule of the invention or a vector of the invention. This is preferably a bacterial cell or a plant cell.

It was now found that the protein encoded by the nucleic acid molecules of the invention influences the starch synthesis or modification and that changes in the amount of the protein in plant cells lead to changes in the starch metabolism of the plant, especially to the synthesis of starch with modified physical and chemical properties.

By providing the nucleic acid molecules of the invention it is possible to produce plants by means of recombinant DNA techniques synthesizing a modified starch which differs from the starch synthesized in wildtype plants with respect to its structure and its physical and chemical properties. For this purpose, the nucleic acid molecules of the invention are linked to regulatory elements, which ensure the transcription and translation in plant cells, and they are introduced into the plant cells.

Therefore, the present invention also relates to transgenic plant cells containing a nucleic acid molecule of the invention wherein the same is linked to regulatory elements which ensure the transcription in plant cells. The regulatory elements are preferably heterologous with respect to the nucleic acid molecule.

Such plant cells of the invention differ from naturally occurring plants among other things in that at least one copy of the nucleic acid molecule of the invention is integrated in their genome, possibly in addition to the naturally occurring copies. Furthermore, this/these additional copy/copies is/are integrated at a location in the genome at which they do not occur naturally. This may be proved, for example, by means of a Southern Blot analysis. Furthermore, such transgenic plant cells can preferably be distinguished from corresponding naturally occurring plant cells by at least one of the following features: If the nucleic acid molecule according to the invention, which was introduced into the plant cells, is

heterologous to the plant cells, the transgenic cells can be distinguished from non transformed cells due to the presence of transcripts from the introduced molecule according to the invention. Such transcripts can be detected, e.g., by Northern Blot analysis. Preferably the transgenic cells furthermore contain the protein encoded by the nucleic acid molecule according to the invention. The presence of the protein can be detected, e.g., by immunological methods such as Western Blot analysis.

If the nucleic acid molecule according to the invention which was introduced into the cells is homologous with respect to the cells, the transgenic cells can be distinguished from non-transformed cells, for example, due to the additional expression of the nucleic acid molecule according to the invention. In particular, the transgenic cells contain preferably more transcripts of the nucleic acid molecules according to the invention. This can be detected, e.g., by Northern Blot analysis. "More" preferably means at least 10% more, more preferably at least 20% more, and even more preferably at least 50% more. Accordingly, the transgenic cells contain preferably more protein according to the invention in comparison to non-transformed cells. This can be detected, e.g., by Western Blot analysis. Preferably, the cells contain at least 10% more protein according to the invention, more preferably at least 20% and even more preferably at least 50% more.

By means of methods known to the skilled person the transgenic plant cells can be regenerated to whole plants. The plants obtainable by regenerating the transgenic plant cells of the invention are also the subject-matter of the present invention.

A further subject-matter of the invention are plants which contain the above-described transgenic plant cells. The transgenic plants may in principle be plants of any desired

species, i.e. they may be monocotyledonous as well as dicotyledonous plants. These are preferably useful plants, in particular starch-synthesizing or starch-storing plants such as cereals (rye, barley, oats, wheat, millet, sago etc.), rice, maize, peas, wrinkled peas, cassava, potato, tomato, oil seed rape, soy bean, hemp, flax, sunflower, cow-pea and arrowroot.

The present invention also relates to a process for the production of a modified starch comprising the step of extracting from the above-described plants according to the invention and/or from starch storing parts of such plants the starch. Preferably, such a process furthermore comprises the steps of cultivating plants according to the invention and harvesting the cultivated plants and/or starch storing parts of these plants before the extraction of the starch.

Methods for extracting starch from plants or from starch storing parts of plants are well known to the person skilled in the art. Methods to extract starch from maize seeds are described, for example, in Eckhoff et al. (Cereal Chem. 73 (1996), 54-57). Extraction of maize starch on an industrial scale is normally achieved by "wet-milling". Furthermore, methods for the extraction of starch from various starch storing plants are described, for example, in Starch: Chemistry and Technology (eds.: Whistler, BeMiller and Paschall (1994) 2nd Edition, Academic Press Inc. London LTD; ISBN 0-12-746270-8; see e.g. Chapter XII, page 417-468: Corn and Sorghum Starches: Production; by Watson, S.A.; Chapter XIII, page 469-479: Tapioca, Arrowroot and Sago Starches: Production; by Corbishley and Miller; Chapter XIV, page 479-490: Potato Starch: Production and Uses; by Mitch; Chapter XV, page 491-506: Wheat starch: Production, Modification and Uses; by Knight and Olson; and Chapter XVI, page 507-528: Rice starch: Production and Uses; by Rohwer and Klem). Means usually used in

methods for the extraction of starches from plant materials are separators, decanters, hydroclones and different kinds of machines for drying the starch, e.g., spray drier or jet drier.

The present invention also relates to the starch obtainable from the transgenic plant cells and plants of the invention or by the above described process. Due to the expression or the additional expression of a nucleic acid molecule of the invention, the transgenic plant cells and plants of the invention synthesize a starch which is modified when compared to starch from wildtype-plants, i.e. non-transformed plants. In particular, such a starch has preferably a higher phosphate content than starch synthesized by corresponding non-transformed cells or plants. A higher phosphate content preferably means that the starch contains at least 10% more phosphate, more preferably at least 30%, even more preferably at least 50% and particularly preferred at least 100% more phosphate than starch from corresponding non-transformed cells or plants. Starches with a high content of phosphate are, for example, of particular interest for the paper industry, e.g., for the preparation of the surface of paper. Normally, the paper industry uses chemically modified starch, for example, hydroxyethylated or phosphorylated starch, for the surface sizing or coating. The production of highly phosphorylated starch in plants would thus avoid the necessity to chemically modify starch in order to adapt it to the requirements of the paper industry.

A further subject-matter of the present invention is a method for the production of a protein which is present in plant cells in granule-bound form as well as in soluble from, in which host cells of the invention are cultivated under conditions that allow for the expression of the protein and in which the

protein is then isolated from the cultivated cells and/or the culture medium.

Furthermore, the invention relates to proteins encoded by the nucleic acid molecules of the invention as well as to proteins obtainable by the above-described method. These are preferably proteins from maize encoded by nuclear genes and which are localized in the plastids. In the plastids these enzymes are present in granule-bound as well as in free form.

A further subject-matter of the invention are antibodies which specifically recognize a protein of the invention. These may be monoclonal as well as polyclonal antibodies. Methods for the production of such antibodies are known to the skilled person.

Furthermore, the present invention relates to nucleic acid molecules specifically hybridizing with a nucleic acid molecule of the invention and exhibiting a length of at least 15 nucleotides. In this context specifically hybridizing signifies that under conventional hybridization conditions, preferably under stringent conditions, cross-hybridization with sequences encoding other proteins do not significantly occur. Such nucleic acid molecules preferably have a length of at least 20, more preferably a length of at least 50 and most preferably a length of at least 100 nucleotides. Such molecules can be used, for example, as PCR primers, as hybridization probes or as DNA molecules which encode antisense RNA.

Furthermore, it was found that it is possible to influence the properties of the starch synthesized in plant cells by reducing the amount of proteins encoded by the nucleic acid molecules according to the invention in the cells. This reduction may be effected, for example, by means of antisense expression of the

nucleic acid molecules of the invention, expression of suitable ribozymes or cosuppression.

Therefore, DNA molecules encoding an antisense RNA which is complementary to transcripts of a DNA molecule of the invention are also the subject-matter of the present invention, as well as these antisense molecules. Thereby, complementary does not signify that the encoded RNA has to be 100% complementary. A low degree of complementarity is sufficient, as long as it is high enough in order to inhibit the expression of a protein of the invention upon expression in plant cells. The transcribed RNA is preferably at least 90% and most preferably at least 95% complementary to the transcript of the nucleic acid molecule of the invention. In order to cause an antisense-effect during the transcription in plant cells such DNA molecules have a length of at least 15 bp, preferably a length of more than 100 bp and most preferably a length of more than 500 bp, however, usually less than 5000 bp, preferably shorter than 2500 bp.

The invention further relates to DNA molecules which, during expression in plant cells, lead to the synthesis of an RNA which in the plant cells due to a cosupression-effect reduces the expression of the nucleic acid molecules of the invention encoding the described protein. The invention also relates to RNA molecules encoded thereby. The principle of the cosupression as well as the production of corresponding DNA sequences is precisely described, for example, in WO 90/12084. Such DNA molecules preferably encode a RNA having a high degree of homology to transcripts of the nucleic acid molecules of the invention. It is, however, not absolutely necessary that the coding RNA is translatable into a protein.

In a further embodiment the present invention relates to DNA molecules encoding an RNA molecule with ribozyme activity which

specifically cleaves transcripts of a DNA molecule of the invention as well as these encoded RNA molecules.

Ribozymes are catalytically active RNA molecules capable of cleaving RNA molecules and specific target sequences. By means of recombinant DNA techniques it is possible to alter the specificity of ribozymes. There are various classes of ribozymes. For practical applications aiming at the specific cleavage of the transcript of a certain gene, use is preferably made of representatives of two different groups of ribozymes. The first group is made up of ribozymes which belong to the group I intron ribozyme type. The second group consists of ribozymes which as a characteristic structural feature exhibit the so-called "hammerhead" motif. The specific recognition of the target RNA molecule may be modified by altering the sequences flanking this motif. By base pairing with sequences in the target molecule these sequences determine the position at which the catalytic reaction and therefore the cleavage of the target molecule takes place. Since the sequence requirements for an efficient cleavage are low, it is in principle possible to develop specific ribozymes for practically each desired RNA molecule.

In order to produce DNA molecules encoding a ribozyme which specifically cleaves transcripts of a DNA molecule of the invention, for example a DNA sequence encoding a catalytic domain of a ribozyme is bilaterally linked with DNA sequences which are homologous to sequences of the target enzyme. Sequences encoding the catalytic domain may for example be the catalytic domains of the satellite DNA of the SCMo virus (Davies et al., Virology 177 (1990), 216-224) or that of the satellite DNA of the TobR virus (Steinecke et al., EMBO J. 11 (1992), 1525-1530; Haseloff and Gerlach, Nature 334 (1988), 585-591). The DNA sequences flanking the catalytic domain are preferably derived from the above-described DNA molecules of the invention.

In a further embodiment the present invention relates to vectors containing the above-described DNA molecules, in particular those in which the described DNA molecules are linked with regulatory elements ensuring the transcription in plant cells.
Furthermore, the present invention relates to host cells containing the described DNA molecules or vectors. The host cell may be a prokaryotic cell, such as a bacterial cell, or a eukaryotic cell. The eucaryotic host cells are preferably plant cells.

Furthermore, the invention relates to transgenic plant cells containing an above-described DNA molecule encoding an antisense-RNA, a ribozyme or an RNA which leads to a cosuppression effect, whereby the DNA molecule is linked to DNA elements ensuring the transcription in plant cells. These transgenic plant cells may be regenerated to whole plants according to well-known techniques. Thus, the invention also relates to plants which may be obtained through regeneration from the described transgenic plant cells, as well as to plants containing the described transgenic plant cells. The transgenic plants themselves may be plants of any desired plant species, preferably useful plants, particularly starch-storing ones, as indicated above, and most preferably maize plant cells.

Furthermore, the invention relates to the antisense RNA molecules encoded by the described DNA molecules, as well as to RNA molecules with ribozyme activity and RNA molecules which lead to a cosupression effect which are obtainable, for example, by means of transcription.

A further subject-matter of the invention is a method for the production of transgenic plant cells, which in comparison to

non-transformed cells synthesize a modified starch. In this method the amount of proteins encoded by the DNA molecules of the invention, which are present in the cells in endogenic form, is reduced in the plant cells.

In a preferred embodiment this reduction is effected by means of an antisense effect. For this purpose the DNA molecules of the invention or parts thereof are linked in antisense orientation with a promoter ensuring the transcription in plant cells and possibly with a termination signal ensuring the termination of the transcription as well as the polyadenylation of the transcript. In order to ensure an efficient antisense effect in the plant cells the synthesized antisense RNA should exhibit a minimum length of 15 nucleotides, preferably of at least 100 nucleotides and most preferably of at least 500 nucleotides. Furthermore, the DNA sequence encoding the antisense RNA should be homologous with respect to the plant species to be transformed. However, DNA sequences exhibiting a high degree of homology to DNA sequences which are present in the cells in endogenic form may also be used, preferably with an homology of more than 90% and most preferably with an homology of more than 95%.

In a further embodiment the reduction of the amount of proteins encoded by the DNA molecules of the invention is effected by a ribozyme effect. The basic effect of ribozymes as well as the construction of DNA molecules encoding such RNA molecules have already been described above. In order to express an RNA with ribozyme activity in transgenic cells the above described DNA molecules encoding a ribozyme are linked with DNA elements which ensure the transcription in plant cells, particularly with a promoter and a termination signal. The ribozymes synthesized in the plant cells lead to the cleavage of

transcripts of DNA molecules of the invention which are present in the plant cells in endogenic form.

A further possibility in order to reduce the amount of proteins encoded by the nucleic acid molecules of the invention is cosupression. Therefore, the plant cells obtainable by the method of the invention are a further subject matter. These plant cells are characterized in that their amount of proteins encoded by the DNA molecules of the invention is reduced and that in comparison to wildtype cells they synthesize a modified starch.

Preferably, the transgenic cells show a reduction in the amount of transcripts encoding a protein according to the present invention of at least 30%, more preferably of at least 50%, even more preferably of at least 70% and most preferably of at least 90% in comparison to corresponding non-transformed cells. The amount of transcripts can be determined, for example, by Northern Blot analysis. Furthermore, the cells preferably show a corresponding reduction of the amount of the protein according to the invention. This can be determined, for example, by immunological methods such as Western Blot analysis.

In a particularly preferred embodiment of the present invention not only the synthesis of a protein of the invention is reduced in the transformed plant cells, but moreover also the synthesis of at least one further enzyme involved in starch synthesis and/or modification. In this context, for example, starch granule-bound starch synthases or branching enzymes are preferred.

Furthermore, the invention relates to plants obtainable by regeneration of the described plant cells as well as to plants containing the described cells of the invention.

The present invention also relates to a process for the production of a modified starch comprising the step of extracting from the above-described plants according to the invention and/or from starch storing parts of such plants the starch. Preferably, such a process furthermore comprises the steps of cultivating plants according to the invention; and harvesting the cultivated plants and/or starch storing parts of these plants before the extraction of the starch.

The present invention also relates to the starch obtainable from the described transgenic plant cells and plants or obtainable by the above described process. Due to the expression of the described DNA molecules encoding antisense RNA, a ribozyme or a cosupression RNA in the transgenic plant cells the amount of proteins encoded by the DNA molecules of the invention which are present in the cells in endogenic form, is reduced. Surprisingly, this reduction leads to a drastic change of the physical and chemical properties of the starch synthesized in the plant cells. When compared to starch from non-transformed cells or plants the modified starch preferably exhibits altered pastification properties, i.e. an altered viscosity of the watery solutions of the starch and/or an altered, in particular a reduced phosphate content.

The expression of the nucleic acid molecules of the invention may in principle take place in any kind of plant species. Monocotyledonous and dicotyledonous plants are preferred, in particular useful plants and preferably starch-storing plants such as cereals (rye, barley, oats, wheat, millet, sago etc.), rice, maize, peas, wrinkled peas, cassava, potato, tomato,

oilseed rape, soy bean, hemp, flax, sunflower, cow-pea and arrowroot.

Within the framework of the present invention the term "regulatory DNA elements ensuring the transcription in plant cells" are DNA regions which allow for the initiation or the termination of transcription in plant cells. DNA regions ensuring the initiation of transcription are in particular promoters.

For the expression of the various above-described DNA molecules of the invention in plants any promoter functioning in plant cells may be used. The promoter may be homologous or heterologous with respect to the used plant species. Use may, for example, be made of the 35S promoter of the cauliflower mosaic virus (Odell et al., Nature 313 (1985), 810-812) which ensures a constitutive expression in all plant tissues and also of the promoter construct described in WO/9401571. However, use may also be made of promoters which lead to an expression of subsequent sequences only at a point of time determined by exogenous factors (such as in WO/9307279) or in a particular tissue of the plant (see e.g. Stockhaus et al., EMBO J. 8 (1989), 2245-2251). Promoters which are active in the starch-storing parts of the plant to be transformed are preferably used. In the case of maize these parts are the maize seeds, in the case of potatoes the tubers. In order to transform potatoes the tuber-specific B33-promoter (Rocha-Sosa et al., EMBO J. 8 (1989), 23-29) may be used particularly, but not exclusively. Apart from promoters, DNA regions initiating transcription may also contain DNA sequences ensuring a further increase of transcription, such as the so-called enhancer-elements.

Furthermore, the term "regulatory DNA elements" may also comprise termination signals which serve to correctly end the transcription and to add a poly-A-tail to the transcript which is believed to stabilize the transcripts. Such elements are

described in the literature and can be exchanged as desired. Examples for such termination sequences are the 3'-nontranslatable regions comprising the polyadenylation signal of the nopaline synthase gene (NOS gene) or the octopine synthase gene (Gielen et al., EMBO J. 8 (1989), 23-29) from agrobacteria, or the 3'-nontranslatable regions of the genes of the storage proteins from soy bean as well as the genes of the small subunit of ribulose-1,5-biphosphate-carboxylase (ssRUBISCO).

The introduction of the DNA molecules of the invention into plant cells is preferably carried out using plasmids. Plasmids ensuring a stable integration of the DNA into the plant genome are preferred.

In order to prepare the introduction of foreign genes in higher plants a large number of cloning vectors are at disposal, containing a replication signal for E.coli and a marker gene for the selection of transformed bacterial cells. Examples for such vectors are pBR322, pUC series, M13mp series, pACYC184 etc. The desired sequence may be integrated into the vector at a suitable restriction site. The obtained plasmid is used for the transformation of E.coli cells. Transformed E.coli cells are cultivated in a suitable medium and subsequently harvested and lysed. The plasmid is recovered by means of standard methods. As an analyzing method for the characterization of the obtained plasmid DNA use is generally made of restriction analysis and sequence analysis. After each manipulation the plasmid DNA may be cleaved and the obtained DNA fragments may be linked to other DNA sequences.

In order to introduce DNA into plant host cells a wide range of techniques are at disposal. These techniques comprise the transformation of plant cells with T-DNA by using Agrobacterium tumefaciens or Agrobacterium rhizogenes as transformation medium, the fusion of protoplasts, the injection and the

electroporation of DNA, the introduction of DNA by means of the biolistic method as well as further possibilities.
In the case of injection and electroporation of DNA into plant cells, there are no special demands made to the plasmids used. Simple plasmids such as pUC derivatives may be used. However, in case that whole plants are to be regenerated from cells transformed in such a way, a selectable marker gene should be present.
Depending on the method of introducting desired genes into the plant cell, further DNA sequences may be necessary. If the Ti- or Ri-plasmid is used e.g. for the transformation of the plant cell, at least the right border, more frequently, however, the right and left border of the Ti- and Ri-plasmid T-DNA has to be connected to the foreign gene to be introduced as a flanking region.
If Agrobacteria are used for transformation, the DNA which is to be introduced must be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. Due to sequences homologous to the sequences within the T-DNA, the intermediate vectors may be integrated into the Ti- or Ri-plasmid of the Agrobacterium due to homologous recombination. This also contains the vir-region necessary for the transfer of the T-DNA. Intermediate vectors cannot replicate in Agrobacteria. By means of a helper plasmid the intermediate vector may be transferred to Agrobacterium tumefaciens (conjugation). Binary vectors may replicate in E.coli as well as in Agrobacteria. They contain a selectable marker gene as well as a linker or polylinker which is framed by the right and the left T-DNA border region. They may be transformed directly into the Agrobacteria (Holsters et al. Mol. Gen. Genet. 163 (1978), 181-187). The plasmids used for the transformation of the Agrobacteria further comprise a selectable marker gene, such as the NPT II gene which allows for selecting transformed bacteria. The Agrobacterium acting as host cell should contain

a plasmid carrying a vir-region. The vir-region is necessary for the transfer of the T-DNA into the plant cell. Additional T-DNA may be present. The Agrobacterium transformed in such a way is used for the transformation of plant cells.
The use of T-DNA for the transformation of plant cells was investigated intensely and described sufficiently in EP 120 516; Hoekema, In: The Binary Plant Vector System Offsetdrukkerij Kanters B.V., Alblasserdam (1985), Chapter V; Fraley et al., Crit. Rev. Plant. Sci., 4, 1-46 and An et al. EMBO J. 4 (1985), 277-287. Some binary vectors may already be obtained commercially, such as pBIN19 (Clontech Laboratories, Inc., USA).
For transferring the DNA into the plant cells, plant explants may suitably be co-cultivated with Agrobacterium tumefaciens or Agrobacterium rhizogenes. From the infected plant material (e.g. pieces of leaves, stem segments, roots, but also protoplasts or suspension-cultivated plant cells) whole plants may then be regenerated in a suitable medium which may contain antibiotics or biozides for the selection of transformed cells. The plants obtained in such a way may then be examined as to whether the introduced DNA is present or not. Other possibilities in order to introduce foreign DNA by using the biolistic method or by transforming protoplasts are known to the skilled person (cf. e.g. Willmitzer, L., 1993 Transgenic plants. In: Biotechnology, A Multi-Volume Comprehensive Treatise (H.J. Rehm, G. Reed, A. Pühler, P. Stadler, editors), Vol. 2, 627-659, VCH Weinheim-New York-Basel-Cambridge).

Whereas the transformation of dicotyledonous plants by Ti-plasmid-vector systems by means of Agrobacterium tumefaciens is a well-established method, more recent studies indicate that the transformation with vectors based on Agrobacterium can also be used in the case of monocotyledonous plants (Chan et al.,

Plant Mol. Biol. 22 (1993), 491-506; Hiei et al., Plant J. 6 (1994), 271-282).

Alternative systems for the transformation of monocotyledonous plants are the transformation by means of the biolistic approach, protoplast transformation, electroporation of partially permeablized cells, the introduction of DNA by means of glass fibers.

There are various references in the relevant literature dealing specifically with the transformation of maize (cf. e.g. WO95/06128, EP 0 513 849; EP 0 465 875). In EP 292 435 a method is described by means of which fertile plants may be obtained starting from mucousless, friable granulous maize callus. In this context it was furthermore observed by Shillito et al. (Bio/Technology 7 (1989), 581) that for regenerating fertile plants it is necessary to start from callus-suspension cultures from which a culture of dividing protoplasts can be produced which is capable to regenerate to plants. After an in vitro cultivation period of 7 to 8 months Shillito et al. obtain plants with viable descendants which, however, exhibited abnormalities in morphology and reproductivity.

Prioli and Söndahl (Bio/Technology 7 (1989), 589) have described how to regenerate and to obtain fertile plants from maize protoplasts of the Cateto maize inbreed Cat 100-1. The authors assume that the regeneration of protoplast to fertile plants depends on a number of various factors such as the genotype, the physiological state of the donor-cell and the cultivation conditions. Once the introduced DNA has been integrated in the genome of the plant cell, it usually continues to be stable there and also remains within the descendants of the originally transformed cell. It usually contains a selectable marker which confers resistance against biozides or against an antibiotic such as kanamycin, G 418, bleomycin, hygromycin or phosphinotricine etc. to the transformed plant cells. The individually selected marker

should therefore allow for a selection of transformed cells against cells lacking the introduced DNA.
The transformed cells grow in the usual way within the plant (see also McCormick et al., Plant Cell Reports 5 (1986), 81-84). The resulting plants can be cultivated in the usual way and cross-bred with plants having the same transformed genetic heritage or another genetic heritage. The resulting hybrid individuals have the corresponding phenotypic properties.
Two or more generations should be grown in order to ensure whether the phenotypic feature is kept stably and whether it is transferred. Furthermore, seeds should be harvested in order to ensure that the corresponding phenotype or other properties will remain.

Due to its properties the starch obtained from the plant cells or from the plants of the invention or obtainable by the processes of the invention is not only suitable for the specific purposes already mentioned herein, but also for various industrial uses.
Basically, starch can be subdivided into two major fields. One field comprises the hydrolysis products of starch and the so-called native starches. The hydrolysis products essentially comprise glucose and glucans components obtained by enzymatic or chemical processes. They can be used for further processes, such as fermentation and chemical modifications. In this context, it might be of importance that the hydrolysis process can be carried out simply and inexpensively. Currently, it is carried out substantially enzymatically using amyloglucosidase. It is thinkable that costs might be reduced by using lower amounts of enzymes for hydrolysis due to changes in the starch structure, e.g. increasing the surface of the grain, improved digestibility due to less branching or a steric structure, which limits the accessibility for the used enzymes.

The use of the so-called native starch which is used because of its polymer structure can be subdivided into two further areas:

(a) Use in foodstuffs

Starch is a classic additive for various foodstuffs, in which it essentially serves the purpose of binding aqueous additives and/or causes an increased viscosity or an increased gel formation. Important characteristic properties are flowing and sorption behavior, swelling and pastification temperature, viscosity and thickening performance, solubility of the starch, transparency and paste structure, heat, shear and acid resistance, tendency to retrogradation, capability of film formation, resistance to freezing/thawing, digestibility as well as the capability of complex formation with e.g. inorganic or organic ions.

(b) Use in non-foodstuffs

The other major field of application is the use of starch as an adjuvant in various production processes or as an additive in technical products. The major fields of application for the use of starch as an adjuvant are, first of all, the paper and cardboard industry. In this field, the starch is mainly used for retention (holding back solids), for sizing filler and fine particles, as solidifying substance and for dehydration. In addition, the advantageous properties of starch with regard to stiffness, hardness, sound, grip, gloss, smoothness, tear strength as well as the surfaces are utilized.

Within the paper production process, a differentiation can be made between four fields of application, namely surface, coating, mass and spraying.

The requirements on starch with regard to surface treatment are essentially a high degree of brightness, corresponding viscosity, high viscosity stability, good

film formation as well as low formation of dust. When used in coating the solid content, a corresponding viscosity, a high capability to bind as well as a high pigment affinity play an important role. As an additive to the mass rapid, uniform, loss-free dispersion, high mechanical stability and complete retention in the paper pulp are of importance. When using the starch in spraying, corresponding content of solids, high viscosity as well as high capability to bind are also significant.
A major field of application is, for instance, in the adhesive industry, where the fields of application are subdivided into four areas: the use as pure starch glue, the use in starch glues prepared with special chemicals, the use of starch as an additive to synthetic resins and polymer dispersions as well as the use of starches as extenders for synthetic adhesives. 90% of all starch-based adhesives are used in the production of corrugated board, paper sacks and bags, composite materials for paper and aluminum, boxes and wetting glue for envelopes, stamps, etc.
Another possible use as adjuvant and additive is in the production of textiles and textile care products. Within the textile industry, a differentiation can be made between the following four fields of application: the use of starch as a sizing agent, i.e. as an adjuvant for smoothing and strengthening the burring behavior for the protection against tensile forces active in weaving as well as for the increase of wear resistance during weaving, as an agent for textile improvement mainly after quality-deteriorating pretreatments, such as bleaching, dying, etc., as thickener in the production of dye pastes for the prevention of dye diffusion and as an additive for warping agents for sewing yarns.

Furthermore, starch may be used as an additive in building materials. One example is the production of gypsum plaster boards, in which the starch mixed in the thin plaster pastifies with the water, diffuses at the surface of the gypsum board and thus binds the cardboard to the board. Other fields of application are admixing it to plaster and mineral fibers. In ready-mixed concrete, starch may be used for the deceleration of the sizing process.
Furthermore, the starch is advantageous for the production of means for ground stabilization used for the temporary protection of ground particles against water in artificial earth shifting. According to state-of-the-art knowledge, combination products consisting of starch and polymer emulsions can be considered to have the same erosion- and encrustation-reducing effect as the products used so far; however, they are considerably less expensive.
Another field of application is the use of starch in plant protectives for the modification of the specific properties of these preparations. For instance, starches are used for improving the wetting of plant protectives and fertilizers, for the dosed release of the active ingredients, for the conversion of liquid, volatile and/or odorous active ingredients into microcristalline, stable, deformable substances, for mixing incompatible compositions and for the prolongation of the duration of the effect due to a reduced disintegration.
Starch may also be used in the fields of drugs, medicine and in the cosmetics industry. In the pharmaceutical industry, the starch may be used as a binder for tablets or for the dilution of the binder in capsules. Furthermore, starch is suitable as disintegrant for tablets since, upon swallowing, it absorbs fluid and after a short time it swells so much that the active ingredient is released. For qualitative reasons, medicinal flowance

and dusting powders are further fields of application. In the field of cosmetics, the starch may for example be used as a carrier of powder additives, such as scents and salicylic acid. A relatively extensive field of application for the starch is toothpaste.

The use of starch as an additive in coal and briquettes is also thinkable. By adding starch, coal can be quantitatively agglomerated and/or briquetted in high quality, thus preventing premature disintegration of the briquettes. Barbecue coal contains between 4 and 6% added starch, calorated coal between 0.1 and 0.5%. Furthermore, the starch is suitable as a binding agent since adding it to coal and briquette can considerably reduce the emission of toxic substances.

Furthermore, the starch may be used as a flocculant in the processing of ore and coal slurry.

Another field of application is the use as an additive to process materials in casting. For various casting processes cores produced from sands mixed with binding agents are needed. Nowadays, the most commonly used binding agent is bentonite mixed with modified starches, mostly swelling starches.

The purpose of adding starch is increased flow resistance as well as improved binding strength. Moreover, swelling starches may fulfill more prerequisites for the production process, such as dispersability in cold water, rehydratisability, good mixability in sand and high capability of binding water.

In the rubber industry starch may be used for improving the technical and optical quality. Reasons for this are improved surface gloss, grip and appearance. For this purpose, the starch is dispersed on the sticky rubberized surfaces of rubber substances before the cold

vulcanization. It may also be used for improving the printability of rubber.

Another field of application for the modified starch is the production of leather substitutes.

In the plastics market the following fields of application are emerging: the integration of products derived from starch into the processing process (starch is only a filler, there is no direct bond between synthetic polymer and starch) or, alternatively, the integration of products derived from starch into the production of polymers (starch and polymer form a stable bond).

The use of the starch as a pure filler cannot compete with other substances such as talcum. This situation is different when the specific starch properties become effective and the property profile of the end products is thus clearly changed. One example is the use of starch products in the processing of thermoplastic materials, such as polyethylene. Thereby, starch and the synthetic polymer are combined in a ratio of 1 : 1 by means of coexpression to form a 'master batch', from which various products are produced by means of common techniques using granulated polyethylene. The integration of starch in polyethylene films may cause an increased substance permeability in hollow bodies, improved water vapor permeability, improved antistatic behavior, improved anti-block behavior as well as improved printability with aqueous dyes.

Another possibility is the use of the starch in polyurethane foams. Due to the adaptation of starch derivatives as well as due to the optimization of processing techniques, it is possible to specifically control the reaction between synthetic polymers and the starch's hydroxy groups. The results are polyurethane films having the following property profiles due to the use of starch: a reduced coefficient of thermal expansion, decreased shrinking behavior, improved pressure/tension behavior, increased water vapor permeability

without a change in water acceptance, reduced flammability and cracking density, no drop off of combustible parts, no halides and reduced aging. Disadvantages that presently still exist are reduced pressure and impact strength.
Product development of film is not the only option. Also solid plastics products, such as pots, plates and bowls can be produced by means of a starch content of more than 50%. Furthermore, the starch/polymer mixtures offer the advantage that they are much easier biodegradable.
Furthermore, due to their extreme capability to bind water, starch graft polymers have gained utmost importance. These are products having a backbone of starch and a side lattice of a synthetic monomer grafted on according to the principle of radical chain mechanism. The starch graft polymers available nowadays are characterized by an improved binding and retaining capability of up to 1000 g water per g starch at a high viscosity. These super absorbers are used mainly in the hygiene field, e.g. in products such as diapers and sheets, as well as in the agricultural sector, e.g. in seed pellets.

What is decisive for the use of the new starch modified by recombinant DNA techniques are, on the one hand, structure, water content, protein content, lipid content, fiber content, ashes/phosphate content, amylose/amylopectin ratio, distribution of the relative molar mass, degree of branching, granule size and shape as well as crystallization, and on the other hand, the properties resulting in the following features: flow and sorption behavior, pastification temperature, viscosity, thickening performance, solubility, paste structure, transparency, heat, shear and acid resistance, tendency to retrogradation, capability of gel formation, resistance to freezing/thawing, capability of complex formation, iodine binding, film formation, adhesive strength, enzyme stability,

digestibility and reactivity. The most remarkable feature is viscosity.

Moreover, the modified starch obtained from the plant cells of the invention may be subjected to further chemical modification, which will result in further improvement of the quality for certain of the above-described fields of application. These chemical modifications are principally known to the person skilled in the art. These are particularly modifications by means of

- acid treatment
- oxidation and
- esterification (formation of phosphate, nitrate, sulphate, xanthate, acetate and citrate starches. Further organic acids may also be used for esterification.)
- formation of starch ethers (starch alkyl ether, O-allyl ether, hydroxylalkyl ether, O-carboxylmethyl ether, N-containing starch ethers, S-containing starch ethers)
- formation of branched starches
- formation of starch graft polymers.

The invention also relates to propagation material of the plants of the invention, such as seeds, fruits, cuttings, tubers or root stocks, wherein this propagation material contains plant cells of the invention.

Deposits

The plasmids produced and/or used within the framework of the present invention have been deposited at the internationally acknowledged deposit "Deutsche Sammlung von Mikroorganismen (DSM)" in Braunschweig, Federal Republic of Germany, according to the requirements of the Budapest treaty for international acknowledgment of microorganism deposits for patenting (deposit number; deposition date):

plasmid pBinAR Hyg	(DSM 9505)	(10/20/94)
plasmid p33-anti-BE	(DSM 6146)	(08/20/90)
plasmid pRL2	(DSM 10225)	(09/04/95)

Description of the figures

Fig. 1 shows the plasmid p35S-anti-RL.

Plasmid structure:

A = fragment A: CaMV 35S promoter, nt 6909-7437 (Franck et al., Cell 21 (1980), 285-294)

B = fragment B: *Asp*718 fragment from pRL1 with a length of approximately 1949 bp
Orientation relative to the promoter: anti-sense
The arrow indicates the direction of the open reading frame.

C = fragment C: nt 11748-11939 of the T-DNA of Ti-plasmid pTiACH5 T-DNA (Gielen et al., EMBO J. 3 (1984), 835-846)

Fig. 2 shows the plasmid pB33-anti-RL

Plasmid structure:

A = fragment A: B33 promoter of the patatin gene B33 from *Solanum tuberosum* (Rocha-Sosa et al., EMBO J. 8 (1989), 23-29)

B = fragment B: *Asp*718 fragment from pRL1 with a length of approximately 1949 bp
Orientation relative to the promoter: anti-sense
The arrow indicates the direction of the open reading frame.

C = fragment C: nt 11748-11939 of the T-DNA of Ti-plasmid pTiACH5 T-DNA (Gielen et al., EMBO J. 3 (1984), 835-846)

Fig. 3 shows a Brabender curve of a watery starch solution, recorded with a Viskograph-E-type Brabender viscograph, which was isolated from non-transformed potato plants of the variety Désirée (see also Example 8).

Thereby signifying:

Thereby signifying:	Drehm.	torque
	[BE]	Brabender unit
	Temp.	temperature
	A	start of pastification
	B	maximum degree of viscosity
	C	start of the 96°C-period
	D	start of cooling-off period
	E	end of cooling-off period
	F	end of the end-50°C period

The blue line indicates the viscosity; the red line stands for temperature.

Fig. 4 shows a Brabender curve of a watery starch solution, recorded with a Viskograph-E-type Brabender viscograph, which was isolated from potato plants transformed with the plasmid p35S-anti-RL (see also Example 8). For the meaning of the abbreviations see Figure 3.

Fig. 5 shows a Brabender curve of a watery solution of starch from potatoes transformed with the plasmid pB33-anti-RL (see also Example 8), recorded with a Viskograph-E-type Brabender viscograph. For the meaning of the abbreviations see Figure 3.

Fig. 6 shows curves of watery solutions of starch isolated from potato plants (see also Example 12), which were recorded with a Rapid Visco Analyser. The red line stands for the temperature; the blue lines 1, 2, 3 and 4 show the viscosities of the following starch solutions:

Line 1: starch isolated from wildtype plants,

Line 2: starch isolated from plants in which only the branching enzyme was inhibited (cf. Example 1 of patent application WO92/14827),

Line 3: starch isolated from plants in which merely the concentration of the proteins of the invention had been reduced (cf. Example 6).

Line 4: starch isolated from plants which had been transformed with the plasmid p35S-anti-RL in combination with the p35SH-anti-BE plasmid (cf. Example 12).

Fig. 7 shows curves of watery solutions of starch isolated from potato plants (see also Example 13), which were recorded with a Rapid Visco Analyser. The red line stands for the temperature; the blue lines 1, 2, 3 and 4 show the viscosities of the following starch solutions:

Line 1: starch isolated from wildtype plants,

Line 2: starch isolated from plants which had solely been transformed with the plasmid pB33-anti-GBSSI (so-called waxy-potato),

Line 3: starch isolated from plants which had been solely transformed with the plasmid p35S-anti-RL (cf. Example 6).

Line 4: starch isolated from plants which had been transformed with the plasmid pB33-anti-RL in combination with the plasmid pB33-anti-GBSSI (cf. Example 13).

Fig. 8 shows the pRL2 plasmid which comprises a full-length cDNA from potato encoding an R1 enzyme.

The Examples illustrate the invention.

Used media and solutions

Elution buffer:	25 mM Tris pH 8,3
	250 mM glycine
Dialysis buffer:	50 mM Tris-HCl pH 7,0
	50 mM NaCl
	2 mM EDTA
	14,7 mM ß-mercaptoethanol
	0,5 mM PMSF
Protein buffer:	50 mM sodium phosphate buffer pH 7,2
	10 mM EDTA
	0,5 mM PMSF
	14,7 mM ß-mercaptoethanol
Lugol solution:	12 g KI
	6 g I_2
	ad 1,8 l with ddH_2O
20 x SSC:	175.3 g NaCl
	88.2 g sodium citrate
	ad 1000 ml with ddH_2O
	ph 7,0 with 10 N NaOH
10 x MEN:	200 mM MOPS
	50 mM sodium acetate
	10 mM EDTA
	pH 7,0
NSEB buffer:	0,25 M sodium phosphate buffer pH 7,2
	7% SDS

1	mM EDTA
1%	BSA (w/v)

YT	8 g Bacto-Yeast extract
	5 g Bacto-Tryptone
	5 g NaCl
	ad 1000 ml with ddH_2O

Protoplast isolation medium (100 ml)

Cellulase Onozuka R S (Meiji Seika, Japan)	800 mg
Pectolyase Y 23	40 mg
KNO_3	200 mg
KH_2PO_4	136 mg
K_2HPO_4	47 mg
$CaCl_2$ $2H_2O$	147 mg
$MgSO_4$ $7H_2O$	250 mg
Bovine serum albumine (BSA)	20 mg
Glucose	4000 mg
Fructose	4000 mg
Sucrose	1000 mg
pH	5,8
Osmolarity	660 mosm.

Protoplast washing solution 1: like protoplast isolating solution, but without cellulase, pectolyase and BSA

Transformation buffers:

a)	Glucose	0,5 M
	MES	0,1 %
	$MgCl_2$ $6H_2O$	25 mM
	pH	5,8
	adjust to 600 mosm.	

b) PEG 6000-solution

Glucose	0,5 M
$MgCl_2$ $6H_2O$	100 mM
Hepes	20 mM
pH	6,5

PEG 6000 is added to the buffer described in b) immediately prior to the use of the solution (40 % w/v PEG). The solution is filtered with a 0,45 µm sterile filter.

W5 solution

$CaCl_2$	125 mM
NaCl	150 mM
KCl	5 mM
Glucose	50 mM

Protoplast culture medium (indicated in mg/l)

KNO_3	3000
$(NH_4)_2SO_4$	500
$MgSO_4$ $7H_2O$	350
KH_2PO_4	400
$CaCl_2$ $2H_2O$	300

Fe-EDTA and trace elements as in the Murashige-Skoog medium (Physiol. Plant, 15 (1962), 473).

m-inosite	100
Thiamine HCl	1,0
Nicotine acid amide	0,5
Pyridoxine HCl	0,5
Glycine	2,0
Glucuronic acid	750
Galacturonic acid	750

Galactose	500
Maltose	500
Glucose	36.000
Fructose	36.000
Sucrose	30.000
Asparagine	500
Glutamine	100
Proline	300
Caseinhydrolysate	500
2,4 dichlorophenoxy acetic acid (2,4-D)	0,5
pH	5,8
Osmolarity	600 mosm.

Buffer A	2x	SSC
	10x	Denhardts solution
	0,1 %	SDS
	5 mM	EDTA
	50 mM	disodium phosphate
	250 µg/ml	herring sperm DNA

In the example the following standard methods were used:

1. Cloning

For cloning in E.coli the vector pBluescriptSK was used.

For plant transformation the gene constructs were cloned into the binary vector pBinAR (Höfgen and Willmitzer, Plant Sci. 66 (1990), 221-230) and B33-Hyg.

2. Bacterial strains

For the Bluescript vector and for the pBinAR and B33-Hyg constructs use was made of the E.coli strain DH5α (Bethesda Research Laboratories, Gaithersburgh, USA).

The transformation of plasmid in potato plants was carried out by means of the Agrobacterium tumefaciens strain C58C1 pGV2260 (Deblaere et al., Nucl. Acids Res. 13 (1985), 4777:4788).

3. Transformation of Agrobacterium tumefaciens

The DNA transfer was carried out by means of direct transformation according to the method of Höfgen & Willmitzer (Nucleic Acids Res. 16 (1988), 9877). The plasmid DNA of transformed Agrobacteria was isolated according to the method of Birnboim & Doly (Nucleic Acids Res. 7 (1979), 1513-1523) and electrophoretically analyzed after suitable restriction cleavage.

4. Transformation of potatoes

Ten small leaves of a sterile potato culture (Solanum tuberosum L. cv. Désirée) injured by a scalpel were treated with 10 ml MS medium (Murashige & Skoog, Physiol. Plant. 15 (1962), 473-497) with 2% sucrose. The medium contained 50 µl of a Agrobacterium tumefaciens overnight-culture grown under selection. After slightly shaking it for 3-5 minutes, another incubation took place in darkness for two days. The leaves were subsequently put on MS medium with 1,6% glucose, 5 mg/l naphtyle acetic acid, 0,2 mg/l benzylaminopurine, 250 mg/l claforan, 50 mg/l kanamycin or 1 mg/l hygromycin B, and 0,80% Bacto Agar for

callus induction. After a one-week incubation at 25°C and 3000 lux the leaves were put on MS-medium with 1,6% glucose, 1,4 mg/l zeatine ribose, 20 mg/l naphtyle acetic acid, 20 mg/l giberellic acid, 250 mg/l claforan, 50 mg/l kanamycin or 3 mg/l hygromycin B and 0,80% Bacto Agar for shoot induction.

5. Transformation of maize

(a) Production of protoplasts of the cell line DSM 6009

Protoplast isolation

2-4 days, preferably 3 days after the last change of medium in a protoplast suspension culture the liquid medium is pumped off and the remaining cells are washed in 50 ml protoplast washing solution 1 and sucked dry once more. 10 ml protoplast isolation medium are added to 2 g of harvested cell mass. The resuspended cells and cell aggregates are incubated at 27 ± 2°C for 4 to 6 hours in the darkness, while shaking it slightly (at 30 to 40 rpm).

Protoplast purification

As soon as the release of at least 1 million protoplasts/ml has taken place (microscopic inspection), the suspension is sifted through a stainless steel or nylon sieve with a mesh size of 200 or 45 µm. The combination of a 100 µm and a 60 µm sieve allows for separating the cell aggregates just as well. The protoplast-containing filtrate is examined microscopically. It usually contains 98 - 99% protoplasts. The rest are undigested single cells. Protoplast preparations with such a degree of

purity are used for transformation experiments without additional gradient centrifugation. The protoplasts are sedimented by means of centrifugation (100 UpM in the swing-out rotor (100 x g, 3 minutes)). The supernatant is abandoned and the protoplasts are resuspended in washing solution 1. The centrifugation is repeated and the protoplasts are subsequently resuspended in the transformation buffer.

(b) Protoplast transformation

The protoplasts resuspended in the transformation buffer are filled in 10 ml portions into 50 ml polyallomer tubes at a titer of 0.5 - 1 x 10^6 protoplasts/ml. The DNA used for transformation is dissolved in Tris-EDTA (TE) buffer solution. 20 µg plasmid DNA is added to each ml protoplast suspension. A plasmid which provides for resistance to phosphinotricine is used as vector (cf. e.g. EP 0 513 849). After the addition of DNA the protoplast suspension is carefully shaken in order to homogenously distribute the DNA in the solution. Immediately afterwards 5 ml PEG solution is added in drops.
By carefully shaking the tubes the PEG solution is distributed homogenously. Afterwards further 5 ml of PEG solution are added and the homogenous mixing is repeated. The protoplasts remain in the PEG solution for 20 minutes at ± 2° C. Afterwards the protoplasts are sedimented by centrifuging for 3 minutes (100g; 1000 Upm). The supernatant is abandoned. The protoplasts are washed in 20 ml W5 solution by careful shaking and are again subjected to

centrifugation. Then they are resuspended in 20 ml protoplast culture medium, centrifuged anew and again resuspended in culture medium. The titer is adjusted to 6 - 8 x 10^5 protoplasts and the protoplasts are cultivated in 3 ml portions in Petri dishes (Ø 60 mm, height 15 mm). The Petri dishes are sealed with parafilm and stored in darkness at 25 ± 2° C.

(c) Protoplast culture

During the first 2 - 3 weeks after the protoplast isolation and transformation the protoplasts are cultivated without adding fresh medium. As soon as the cells regenerated from the protoplasts have developed into cell aggregates with more than 20 to 50 cells, 1 ml of fresh protoplast culture medium, containing sucrose as an osmotic (90 g/l), is added.

(d) Selection of transformed maize cells and plant regeneration

3 - 10 days after adding fresh medium the cell aggregates developed from the protoplasts may be plated on Agar media with 100 mg/l L-phosphinothricine. N6-medium with the vitamins of the protoplast culture medium, 90 g/l sucrose and 1.0 mg/l 2.4D is as suitable as an analogous medium such as a medium with the macro- and micro-nutritive salts of the MS medium (Murashige and Skoog (1962), see above).

The calli developed from stably transformed protoplasts may grow further on the selective medium. After 3 to 5 weeks, preferably 4 weeks the transgenic calli may be transferred to fresh selection medium

which also contains 100 mg/l L-phosphinothricine which, however, does no longer contain auxine. Within 3 to 5 weeks approximately 50% of the transgenic maize calli which had integrated the L-phosphinothricine-acetyl-transferase gene into their genome, start to differentiate into plants on this medium in the presence of L-phosphinothricine.

(e) Growing of transgenic regenerative plants

The embryogenical transformed maize tissue is cultivated on hormone-free N6-medium (Chu C.C. et al., Sci. Sin. 16 (1975), 659) in the presence of 5×10^{-4} M L-phosphinothricine. On this medium maize embryos, which express the phosphinothricine-acetyl-transferase gene (PAT gene) in a sufficiently strong manner, develop into plants. Non-transformed embryos or such with only a very weak PAT activity die down. As soon as the leaves of the in-vitro plants have reached a length of 4 to 6 mm, they may be transferred into soil. After washing off the Agar residues at the roots the plants are planted into a mixture of clay, sand, vermiculite and potting soil with the ratio 3:1:1:1 and adapted to the soil culture at 90 - 100% of relative atmospheric humidity during the first 3 days after planting. The growing is carried out in a climate chamber with a 14 hour light period of approximately 25000 lux at the height of the plant at a day/night temperature of 23 ± 1/17 ± 1° C. The adapted plants are cultivated at an 65 ± 5% atmospheric humidity.

6. Radioactive marking of DNA fragments

The radioactive marking of DNA fragments was carried out by means of a DNA-Random Primer Labeling Kits by Boehringer (Germany) according to the manufacturer's instructions.

7. Northern Blot Analysis

RNA was isolated from leave tissue according to standard protocols. 50 µg of the RNA was separated on an agarose gel (1.5% agarose, 1 x MEN buffer, 16.6% formaldehyde). After the gel run the gel was briefly washed in water. The RNA was transferred to a Hybond N type nylon membrane (Amersham, UK) with 20 x SSC by means of capillary blot. The membrane was subsequently baked in vacuum for two hours at 80°C.
The membrane was prehybridized in NSEB buffer for two hours at 68°C and subsequently hybridized overnight in NSEB buffer in the presence of the radioactively marked probe at 68°C.

8. Plant maintenance

Potato plants were kept in the greenhouse under the following conditions:

light period	16 hours at 25000 lux and 22°C
dark period	8 hours at 15°C
atmospheric humidity	60%

9. Determination of the amylose/amylopectin ratio in starch obtained from potato plants

Starch was isolated from potato plants according to standard methods and the amylose/amylopectin ratio was determined according to the method described by Hovenkamp-Hermelink et al. (Potato Research 31 (1988) 241-246).

10. Determination of glucose, fructose and sucrose

In order to determine the glucose, fructose and/or sucrose content, small pieces of potato tubers (with a diameter of approx. 10 mm) are frozen in liquid nitrogen and subsequently extracted for 30 min at 80°C in 0.5 ml 10mM HEPES, pH 7.5; 80% (vol./vol.) ethanol. The supernatant containing the soluble components is withdrawn and the volume is determined. The supernatant is used for determining the amount of soluble sugars. The quantitative determination of soluble glucose, fructose and sucrose is carried out in a reaction mixture with the following composition:

100.0 mM imidazole/HCl, pH 6.9
1.5 mM $MgCl_2$
0.5 mM $NADP^+$
1.3 mM ATP
10-50 µl sample
1.0 U glucose-6-phosphate dehydrogenase from yeast

The reaction mixture is incubated at room temperature for 5 minutes. The subsequent determination of sugars is carried out by means of standard photometric methods by measuring the absorption at 340 nm after successive adding of

1.0 unit of hexokinase from yeast
(for determining glucose)

1.0 unit of phosphoglucoisomerase from yeast
(for determining fructose)
and
1.0 unit of invertase from yeast
(for determining sucrose).

Example 1

The isolation of starch granule-bound proteins from potato starch

The isolation of starch granule-bound proteins from potato starch is carried out by means of electroelution in an elution appliance which was constructed analogous to the "Model 442 Electro Eluter" (BIORAD Laboratories Inc., USA) but had a considerably greater volume (approx. 200 ml). 25 g dried starch were dissolved in elution buffer (final volume 80 ml). The starch was derived from potatoes which produce an almost amylose-free starch due to the antisense-expression of a DNA sequence encoding the starch granule-bound starch synthases I (GBSS I) from potato. The suspension was heated to 70-80°C in a water bath. Subsequently 72.07 g urea was added (end concentration 8 M) and the volume was filled up to 180 ml with elution buffer. The starch dissolved during permanent stirring and acquired a paste-like consistency. The proteins were electroeluted from the solution overnight by means of the elution appliance (100 V; 50-60 mA). The eluted proteins were carefully removed from the appliance. Suspended particles were removed in a brief centrifugation. The supernatant was dialyzed at 4°C 2 to 3 times for one hour against dialysis buffer. Subsequently, the volume of the protein solution was determined. The proteins were precipitated by adding ammonium sulfate (final concentration 90 %), which was done during

permanent stirring at 0°C. The precipitated proteins were pelleted by centrifugation and resuspended in protein buffer.

Example 2

Identification and isolation of cDNA sequences encoding starch granule-bound proteins

The proteins isolated according to Example 1 were used for the production of polyclonal antibodies from rabbit, which specifically recognize starch granule-bound proteins.
By means of such antibodies a cDNA expression library was subsequently screened for sequences encoding starch granule-bound proteins, using standard methods.
The expression library was produced as follows:
Poly (A^+)-mRNA was isolated from potato tubers of the "Berolina" variety. Starting from the poly (A^+)-mRNA, cDNA was produced according to the Gubler and Hoffmann method (Gene 25 (1983), 263-269), using an Xho I-Oligo d(t)18 primer. This cDNA was cut with Xho I after EcoR I-linker addition and ligated in an oriented manner in a lambda ZAP II vector (Stratagene) cut with EcoR I and Xho I. Approximately 500,000 plaques of a cDNA library constructed in such a way were screened for sequences which were recognized by polyclonal antibodies directed against starch granule-bound proteins.
In order to analyze the phage plaques these were transferred to nitrocellulose filters which had previously been incubated in a 10 mM IPTG solution for 30 to 60 minutes and had subsequently been dried on filter paper. The transfer took place at 37°C for 3 hours. Subsequently, the filters are incubated at room temperature for 30 minutes in block reagent and washed for 5-10 minutes in TBST buffer. The filters were shaken with the polyclonal antibodies directed against starch granule-bound proteins in a suitable dilution for one hour at room

temperature or for 16 hours at 4°C. The identification of plaques expressing a protein which was recognized by the polyclonal antibodies was carried out by means of the "Blotting detection kit for rabbit antibodies RPN 23" (Amersham UK) according to the manufacturer's instructions.

Phage clones of the cDNA library expressing a protein which was recognized by the polyclonal antibodies were further purified by using standard methods.

By means of the in-vivo excision method, E.coli clones were obtained from positive phage clones containing a double-stranded pBluescript plasmid with the corresponding cDNA insertion. After checking the size and the restriction pattern of the insertions a suitable clone, pRL1, was further analyzed.

Example 3

Sequence analysis of the cDNA insertion of the plasmid pRL1

From an E.coli clone obtained according to Example 2 the plasmid pRL1 was isolated and a part of the sequence of its cDNA insertion was determined by standard procedures using the didesoxynucleotide method (Sanger et al., Proc. Natl. Acad. Sci. USA 74 (1977), 5463-5467). The insertion has a length of about 2450 bp. A part of the nucleotide sequence as well as the amino acid sequence derived therefrom is indicated under Seq ID No. 3 and under Seq ID No. 4.

A sequence analysis and a sequence comparison with known DNA sequences showed that the sequence indicated under Seq ID No. 3 is new and exhibits no significant homology to DNA sequences known so far. Moreover, the sequence analysis showed that the cDNA insertion is only a partial cDNA in which a part of the coding region at the 5'-end is missing.

Example 4

Identification and isolation of a complete cDNA encoding a starch granule-bound protein from Solanum tuberosum

In order to isolate a complete cDNA corresponding to the partial cDNA insertion of the plasmid pRL1, a further cDNA library was produced. This was a guard-cell-specific cDNA library from Solanum tuberosum which was constructed as follows:

At first epidermis fragments from leaves of "Desirée" variety potato plants were produced essentially according to the Hedrich et al. method (Plant Physiol. 89 (1989), 148), by harvesting approximately 60 leaves of six-weeks-old potato plants kept in the greenhouse. The center nerve was removed from the leaves. The leaves were subsequently crushed in a big "Waring blender" (with a volume of 1 liter) four times in cooled, distilled H_2O on the highest level for 15 seconds each. The suspension was filtered through a nylon sieve with a mesh size of 220 µm (Nybolt, Zurich, Switzerland) and washed in cold distilled water several times. The suspension itself was filtered through a 220 µm nylon sieve and intensely washed with cold distilled water. The residues (epidermis fragments) were crushed in a smaller "Waring blender" (with a volume of 250 ml) four times in distilled water and ice on a lower level for 15 seconds each. The suspension was filtered through a 220 µm nylon sieve and washed intensely with cold distilled water. The epidermis fragments (residues) were microscopically examined for contamination by mesophyl cells. If contamination occurred the crushing step was repeated in a small "Waring blender".

The disruption of the guard cells of the epidermis fragments was carried out by means of pulverizing in liquid nitrogen in a cooled mortar for approximately two hours. In order to examine

the disruption of the guard cells, probes were regularly taken and microscopically examined. After two hours, or if a sufficiently high amount of guard cells had been disrupted, the obtained powder was filled into a reaction tube (with a volume of 50 ml) and resuspended in one volume GTC buffer (Chirgwin et al., Biochem. 18 (1979), 5294-5299). The suspension was centrifuged and the supernatant was filtered through Miracloth (Calbiochem, La Jolla, California). The filtrate was subjected to ultracentrifugation for 16 hours, as described in Glisin et al. (Biochemistry 13 (1974), 2633-2637) and Mornex et al. (J. Clin. Inves. 77 (1986), 1952-1961). After the centrifugation the RNA precipitate was dissolved in 250 µl GTC buffer. The RNA was precipitated by adding 0.05 volumes of 1 M acetic acid and 0.7 volumes of ethanol. The RNA was precipitated by centrifugation and the precipitate was washed with 3 M sodium acetate (pH 4.8) and 70% ethanol. The RNA was briefly dried and dissolved in DEPC treated water.

Poly A^+-RNA was isolated from the isolated RNA according to standard methods. Starting from the poly(A^+)-mRNA, cDNA was produced according to the Gubler and Hoffmann method (Gene 25 (1983), 263-269) by means of a Xho I-oligo $d(t)_{18}$ primer. This cDNA was cut with Xho I after EcoR I-linker addition and ligated in an oriented manner in a lambda ZAP II vector (Stratagene GmbH, Heidelberg, Germany) cut with EcoR I and Xho I. The packaging in phage heads was carried out using the Gigapack II Gold kit (Stratagene GmbH, Heidelberg, Germany) according to the manufacturer's instructions.

From such a cDNA library phage clones hybridizing with the cDNA insertion of the pRL1 plasmid were isolated and purified according to standard methods. By means of the in vivo excision method E.coli clones were obtained from positive phage clones containing a double-stranded pBluescript plasmid with the corresponding cDNA insertion. After checking the size and the restriction pattern of the insertions, suitable clones were

subjected to restriction mapping and sequence analysis. From a suitable clone the plasmid pRL2 (DSM 10225) was isolated which contains a complete cDNA which encodes a starch granule-bound protein from potato.

Example 5

Sequence analysis of the cDNA insertion of the pRL2 plasmid

The nucleotide sequence of the cDNA insertion of the pRL2 plasmid was determined as described in Example 3. The insertion has a length of 4856 bp. The nucleotide sequence as well as the amino acid sequence derived therefrom is indicated in Seq ID No. 1 and/or Seq ID No. 2. In the following, the corresponding gene will be called RL-gene. The protein encoded by the coding region will be called R1 enzyme.

Example 6

The construction of the plasmid p35S-anti-RL and the introduction of the plasmid into the genome of potato plants

By means of the restriction endonuclease Asp718 a DNA fragment with an approximate length of 1800 bp was isolated from the pRL1 plasmid. This corresponds to the DNA sequence indicated under Seq ID No. 3 and contains a part of the open reading frame. The fragment was ligated into the binary vector pBinAR cut with Asp718 (Höfgen and Willmitzer, Plant Sci. 66 (1990), 221-230). This is a derivative of the binary vector pBin19 (Bevan, Nucl. Acids Res. 12 (1984), 8711-8721). pBinAR was constructed as follows:
A fragment with a length of 529 bp comprising the nucleotides 6909-7437 of the 35S promoter of the cauliflower-mosaic virus

(Franck et al., Cell 21 (1980), 285-294) was isolated from the plasmid pDH51 (Pietrzak et al., Nucl. Acids Res. 14, 5857-5868) as an EcoR I/Kpn I fragment and ligated between the EcoR I and the Kpn I sites of the pBin19 polylinker. This led to the plasmid pBin19-A.
By means of the restriction endonucleases Pvu II and Hind III a fragment with a length of 192 bp was isolated from the plasmid pAGV40 (Herrera-Estrella et al., Nature 303, 209-213) comprising the polyadenylation signal of gene 3 of the T-DNA of the Ti-plasmid pTiACH5 (Gielen et al., EMBO J. 3, 835-846) (nucleotides 11749-11939). After the addition of Sph I-linkers to the Pvu I site the fragment was ligated between the Sph I and Hind III sites of pBin19-A. This led to plasmid pBinAR.
By means of restriction and sequence analysis recombinant vectors were identified in which the DNA fragment is inserted in the vector in such a way that a part of the coding region of the cDNA insertion from pRL1 is linked with the 35S promoter in antisense orientation. The resulting plasmid p35S-anti-RL is shown in Figure 1.
By inserting the cDNA fragment an expression cassette is produced which consists of the fragments A, B and C:
Fragment A (529 bp) contains the 35S promoter of the cauliflower-mosaic virus (CaMV). The fragment comprises the nucleotides 6909 to 7437 of the CaMV (Franck et al., Cell 21 (1980), 285-294).
Apart from flanking regions, fragment B contains a part of the protein encoding cDNA insertion from plasmid pRL1. This was isolated as an Asp718 fragment of pRL1 as described above and fused to the 35S promoter in antisense orientation.
Fragment C (192 bp) contains the polyadenylation signal of gene 3 of the T-DNA of the Ti-plasmid pTiACH5 (Gielen et al., EMBO J. 3 (1984), 835-846).
The plasmid p35S-anti-RL has a size of approximately 12.8 kb.

The plasmid was transferred into potato plants by means of Agrobacteria-mediated transformation, as described above. From the transformed cells whole plants were regenerated. The transformed plants were cultivated under greenhouse conditions. By analyzing total RNA in a Northern Blot analysis concerning the disappearance of the transcripts complementary to the cDNA, the success of the genetic modification of the plants was assessed. For this purpose, total RNA was isolated from leaves of transformed plants according to standard methods and subsequently separated electrophoretically on an agarose gel. Then it was transferred onto a nylon membrane and hybridized with a radioactively labelled probe having the sequence indicated under Seq ID No. 1 or a part thereof. In about 5-10% of the transformed plants the band indicating the specific transcript under Seq ID No. 1 was missing in the Northern Blot Analysis. The plants were used for analyzing the starch quality.

Example 7

The construction of the plasmid pB33-anti-RL and the introduction of the plasmid into the genome of potato plants

By means of the restriction endonuclease Asp718, a DNA fragment with an approximate length of 1800 bp, which comprises a part of the open reading frame of the cDNA insertion was isolated from the plasmid pRL1 and was ligated into the vector B33-Hyg which was cut with Asp718. This vector was constructed as follows:
The 35S promoter was removed from the pBinAR Hyg vector (DSM 9505) by means of the restriction endonucleases EcoR I and Asp718. A fragment with a length of about 1526 bp comprising the B33 promoter was isolated from the plasmid p33-anti-BE (DSM

6146) by means of EcoR I and Asp718 and inserted into the pBinAR Hyg vector (DSM 9505) cut with EcoR I and Asp718.

By inserting the cDNA fragment into the Asp718 site of the B33-Hyg plasmid, an expression cassette is produced which consists of the fragments A, B and C as follows (Figure 4):

Fragment A contains the B33 promoter from Solanum tuberosum (EP 3775 092; Rocha-Sosa et al., EMBO J. 8 (1989), 23-29).

Apart from flanking regions, fragment B contains a part of the protein encoding region of the cDNA insertion from the pRL1 plasmid. This was isolated as an Asp718 fragment from pRL1 as described above and fused to the 35S promoter in antisense orientation.

Fragment C (192 bp) contains the polyadenylation signal of gene 3 of the T-DNA of the Ti-plasmid pTiACH5 (Gielen et al., EMBO J. 3 (1984), 835-846).

The plasmid pB33-anti-RL has a size of approximately 12.8 kb.

The plasmid was transferred into potato plants by means of Agrobacteria-mediated transformation, as described above. From the transformed cells whole plants were regenerated. The transformed plants were cultivated under greenhouse conditions.

By analyzing total RNA in a Northern Blot analysis concerning the disappearance of the transcripts complementary to the cDNA the success of the genetic modification of the plants was assessed. For this purpose, total RNA was isolated from leaves of transformed plants according to standard methods and subsequently separated electrophoretically on an agarose gel. Then it was transferred onto a nylon membrane and hybridized with a radioactively labelled probe showing the sequence indicated under Seq ID No. 1 or a part thereof. In about 5-10% of the transformed plants the band indicating the transcript hybridizing with the cDNA of the invention was missing in the Northern Blot Analysis. From these plants starch was isolated from tubers and analyzed as described in Example 8.

Example 8

Analysis of the transformed potato plants

The potato plants transformed according to Example 6 and Example 7 were examined with regard to the properties of the synthesized starch. Analyses were carried out with various lines of the potato plants which had been transformed with the plasmid p35S-anti-RL or the plasmid pB33-anti-RL and which in Northern Blot analysis had not exhibited the band indicating transcripts hybridizing to the DNA sequences of the invention.

a) Determination of the viscosity of watery solutions of the starch

In order to determine the viscosity of the watery solutions of the starch synthesized in transformed potato plants, starch was isolated from tubers of plants which had been transformed with the plasmid p35S-anti-RL or the plasmid pB33-anti-RL using standard methods. 30 g of starch were each taken up in 450 ml H_2O and used for analysis in an E viscograph (Brabender OHG Duisburg (Germany)). The appliance was used according to the manufacturer's instructions. In order to determine the viscosity of the watery solution of the starch, the starch suspension was first heated from 50°C to 96°C at a speed of 3°C per minute. The temperature was subsequently kept at 96°C for 30 min. The solution was then cooled from 96°C to 50°C at a speed of 3°C per minute. During the whole process the viscosity was determined. Representative results of such measurements are set forth in the form of graphs in Figures 3, 4 and 5, in which the viscosity is

shown depending on time. Figure 3 shows a typical Brabender graph for starch isolated from wildtype-plants of the potatoe variety Désirée. Figures 4 and 5 show a typical Brabender graph for starch isolated from potato plants which had been transformed with the plasmid p35S-anti-RL or pB33-anti-RL. From these graphs characteristic values may be deduced.

The characteristic values for wildtype-plants are as follows:

Table 1

Value	Time [min : sec]	Torque [BE]	Temperature [°C]
A	6 : 30	60.5 ± 17.7	69.9 ± 0.57
B	11 : 30	1838.0 ± 161.2	86.0 ± 2.1
C	15 : 15	1412.0 ± 18.4	96.0
D	45 : 15	526.0 ± 17.0	96.0
E	60 : 30	812.0 ± 8.5	50.0
F	70 : 45	853.0 ± 5.7	50.0

The values represent the average values obtained from two different measurements.

In Table 1 and the following Tables 2 and 3 the abbreviations signify the following:

A: start of pastification
B: maximum viscosity
C: start of 96°C-period
D: start of cooling-off time
E: end of cooling-off time
F: end of the end-50°C period

For plants which had been transformed with the plasmid p35S-anti-RL (line P2), the characteristic values are the following:

Table 2

Value	Time [min : sec]	Torque [BE]	Temperature [°C]
A	6 : 00	50.0	69.0
B	14 : 00	820.0	93.0
C	15 : 15	815.0	96.0
D	45 : 15	680.0	96.0
E	60 : 30	1150.0	50.0
F	70 : 45	1200.0	50.0

For plants which had been transformed with the plasmid pB33-anti-RL (line P3), the characteristic values are the following:

Table 3

Value	Time [min : sec]	Torque [BE]	Temperature [°C]
A	7:0	31.0	71.0
B	12:45	671.0	88.3
C	15:15	662.0	96.0
D	45:15	607.0	96.0
E	60:30	1063.0	50.0
F	70:45	1021.0	50.0

Figures 3, 4 and 5 explicitly show that the starch obtained from transformed plants differs from starch from wildtype plants particularly in that the viscosity increases only very slightly during heating. Thus, during heating the maximum viscosity of the modified starch from transformed plants is more than 50% lower than in the case of wildtype starch.

During cooling, on the other hand, the viscosity of the starch isolated from transformed plants increases more than in the case of wildtype-plants.

b) Determination of the phosphate content of the starch

The phosphate content of the starch was determined by measuring the amount of phosphate bound to the C-6-position of the glucose residues. For this purpose, starch was first degraded by acid hydrolysis and the glucose-6-phosphate content was subsequently determined by means of an enzyme test, as described in the following.

100 mg starch were incubated in 500 µl 0.7 N HCl for 4 hours at 100°C. After acid hydrolysis 10 µl of the reaction were added to 600 µl imidazole buffer (100 mM imidazole, 5 mM $MgCl_2$, pH 6.9, 0.4 mM NAD^+). The amount of glucose-6-phosphate in the reaction mixture was determined by conversion with the enzyme glucose-6-phosphate-dehydrogenase. For this purpose, 1 U glucose-6-phosphate-dehydrogenase (from Leuconostoc mesenteroides (Boehringer Mannheim)) was added to the reaction mixture and the amount of produced NADH was determined by measuring the absorption at 340 nm.

The glucose-6-phosphate content of 1 mg starch is indicated in the following table for non-transformed potato plants of the variety Désirée as well as for two lines (P1 (35S-anti-RL); P2(35S-anti-RL)) of transgenic potato plants which had been transformed with the plasmid p35S-anti-RL.

Table 4

Plants	nmol glucose-6-phosphate/mg starch	%
Wildtype	12.89 ± 1.34	100
P1 (35S-anti-RL)	2.25 ± 0.41	17.4
P2 (35S-anti-RL)	1.25 ± 0	9.7

The following table shows the glucose-6-phosphate content per milligram starch in potato plants which were transformed with the plasmid pB33-anti-RL, compared to starch from non-transformed plants (S. tuberosum c.v. Désirée).

Table 5

Plants	nmol glucose-6-phosphate/mg starch	%
Wildtype	9.80 ± 0.68	100
7	4.50 ± 0.73	45.9
37	2.64 ± 0.99	26.9
45	1.14 ± 0.44	11.6
31	1.25 ± 0.49	12.8

The plants 7, 37, 45 and 31 represent independent transformants which had been transformed with the plasmid pB33-anti-RL. Plant 37 represents line P3 for which a Brabender graph is plotted in Figure 5.

The values show that the phosphate content of the modified starch from transgenic potato plants is at least 50% lower when compared to starch from wildtype plants.

c) Determination of glucose, fructose and sucrose content of tubers after storage at 4°C

Tubers of plants from various transgenic lines which had been transformed with the antisense-construct p35S-anti-RL

as well as tubers of wildtype plants were stored at 4°C or, respectively, at 20°C in darkness, for two months. Subsequently, the amounts of glucose, fructose and sucrose were determined. For two transgenic lines the representative values obtained were the following:

Table 6

	Glucose		Fructose		Sucrose	
	20°C	4°C	20°C	4°C	20°C	4°C
Wildtype cv Désirée	0.84	55.4	0.62	52.8	8.5	13.1
Transgenic line 15	1.12	6.7	0.75	7.8	7.5	10.1
Transgenic line 11	1.00	6.4	0.75	7.5	6.9	6.9

The values in the table are indicated in µmol hexose or sucrose/g fresh weight.

From the values of Table 6 it becomes obvious that the accumulation of reducing sugars in the tubers is considerably lower in transgenic plants stored at 4°C than in wildtype plants.

Altogether the modified starch isolated from transgenic potato plants resembles starch from maize-wildtype plants. However, in comparison it has the advantage that its taste is neutral and that it is therefore more suitable for various uses in the foodstuffs area.

Example 9

Expression of the cDNA insertion of the pRL2 plasmid in E.coli

(a) Transformation of bacterial cells

In order to express the cDNA insertion of the plasmid pRL2 the cells of the E.coli strain DH5α are first transformed with the pACAC plasmid. This plasmid contains a DNA fragment encoding the ADP-glucose-pyrophosphorylase (AGPase) from E.coli, under the control of the lac Z promoter. The fragment had been isolated from the vector pEcA-15 as a DraI/HaeII fragment with a size of about 1.7 kb (see B. Müller-Röber (1992), dissertation, FU Berlin) and after filling in its sticky ends it had been cloned into a pACAC184 vector linearized with HindIII. The expression of AGPase is to cause an increase of the glycogen synthesis in transformed E.coli cells. The cells transformed in such a way will in the following be named E.coli-K1-cells.

In order to determine the enzyme activity of the protein encoded by the cDNA of plasmid pRL2, E.coli-K1-cells were transformed with the pRL2 plasmid. The transformed E.coli cells which contain the pACAC plasmid as well as the pRL2 plasmid will in the following be named E.coli-K2-cells.

The transfer of the plasmid DNA into the bacterial cells was carried out according to the Hanahan method (J. Mol. Biol. 166 (1983), 557-580). The transformed E.coli cells were plated onto agar culture dishes with the following composition:

YT medium containing

1,5% Bacto agar

50 mM	sodium phosphate buffer, pH 7.2
1%	glucose
10 µg/ml	chloramphenicol in the case of E.coli-K1-cells
or	
10 µg/ml	chloramphenicol and
10 µg/ml	ampicillin in the case of E.coli-K2-cells.

Escherichia coli cells of the DH5α strain which had been transformed with the plasmid pRL2 + pACAC (E.coli-K2-cells) and also - for control - solely with the pACAC plasmid (E.coli-K1-cells), were raised on agar plates. The formed glycogen of the various cultures was examined with respect to the degree of phosphorylization (at the C-6 position of the glucose molecule), as described in the following.

(b) Isolation of bacterial glycogen

In order to isolate bacterial glycogen, the bacteria colony which had grown after transformation was floated from each 6 agar plates (Ø 135 mm) with 5 ml YT medium for each plate. The bacterial suspension was centrifuged at 4500 xg for 5 minutes. The bacterial precipitate was resuspended in 10 ml YT medium. Disruption of the bacteria was carried out by adding 2 volumes of disruption medium (0.2 N NaOH; 1% SDS) and by incubation at room temperature for 5 minutes. By adding 3 volumes of EtOH abs., incubating at 4°C for 30 minutes and subsequent centrifuging at 8000 gx for 15 minutes, the glycogen was sedimented. Then the precipitate was washed with 100 ml of 70% EtOH and again sedimented by means of a centrifugation step (10 minutes at 8000 xg). The washing procedure was repeated four times.

(c) Determination of the total glycogen content

The isolated and sedimented glycogen was first degraded into single glucose molecules by means of acidic hydrolysis (dissolving of the precipitate in 2 ml 0.7 N HCl; incubation for 4 hours at 100°C). The glucose content of the solution was determined by means of coupled enzymatic reaction of a starch test with a photometer (Kontron) at a wave length of 340 nm according to the manufacturer's (Boehringer Mannheim) instructions.
The reaction buffer contains:

100	mM	MOPS, pH 7.5
10	mM	$MgCl_2$
2	mM	EDTA
0.25	mM	NADP
1	mM	ATP
1	U/ml	glucose-6-phosphate-dehydrogenase
2	U/ml	hexokinase

Die measurement was carried out at 25°C with 10 µl glucose solution.

(d) Determination of the glucose-6-phosphate content

In order to determine the content of glucose molecules phosphorylated at the C-6 position, equal amounts of glucose of the various bacterial cultures were used. By adding the same volumes of 0.7 N KOH to the glycogens degraded into its glucose molecules by acidic hydrolysis (as above), the solution was neutralized.

The reaction buffer contains:

100	mM MOPS, pH 7.5
10	mM $MgCl_2$
2	mM EDTA
0.25	mM NADP
2	U/ml glucose-6-phosphate-dehydrogenase

The measurement was carried out at 25°C with 100 to 150 µl glucose solution.

(e) Identification of an enzyme activity phosphorylating bacterial glycogen

The results of the determination of the phosphate content of the glycogen synthesized in the bacterial cells show that the glycogen of the E.coli cells, which had been transformed with the pACAC + pRL2 plasmids, exhibits a 290 ± 25% increased phosphorylation at the C-6 position of the glucose when comparing with the control reaction (E.coli cells transformed with the pACYC) (see the following table).

E.coli cells	glucose-6-phosphase: glucose in glycogen
E.coli-K1	1 : (4600 ± 1150)
E.coli-K2	1 : (1570 ± 390)

The degrees of phosphorylation indicated herein are the average value of at least 6 measurements starting from 6 independent transformations and glycogen isolations.

Example 10

Integration of the plasmid p35S-anti-RL in combination with the plasmid p35SH-anti-BE into the genome of potato plants

The plasmid p35S-anti-RL was constructed as described in Example 6. The plasmid p35SH-anti-BE was constructed as described in the application WO95/07355, Example 3. Both plasmids were sequentially transferred into potato plants by means of the Agrobacterium mediated transformation as described above. For this purpose, the plasmid p35SH-anti-BE was first transformed in potato plants. Whole plants were regenerated and selected for a reduced expression of the branching enzyme gene. Subsequently, the plasmid p35S-anti-RL was transformed into the transgenic plants already showing a reduced expression of the branching enzyme. From the transformed cells transgenic plants were again regenerated and the transformed plants were cultivated under greenhouse conditions. By analyzing total RNA in an RNA Blot analysis with respect to the disappearance of the transcripts complementary to the branching enzyme cDNA or the RL cDNA, the success of the genetic modification of the plants with respect to a highly reduced expression of the branching enzyme gene as well as with respect to a highly reduced expression of the RL gene was assessed. For this purpose, total RNA was isolated from leaves of transformed plants according to the described methods and subsequently separated by means of gel electrophoresis, transferred onto a membrane, hybridized with a radioactively labelled probe showing the sequence indicated under Seq ID No. 1 or a part thereof and then hybridized with a radioactively labelled probe showing the sequence of the branching enzyme cDNA (cf. WO92/14827, Example 1) or a part thereof. In about 5-10% of the transformed plants the band indicating the specific transcript of the sequence indicated under Seq ID No. 1 as well as the

band indicating the specific transcript of the branching enzyme cDNA (cf. WO92/14827) was missing in the RNA Blot Analysis. These plants, which were designated R4 plants were used for analyzing the quality of the starch contained in tubers.

Example 11

Integration of the plasmid pB33-anti-RL in combination with the plasmid pB33-anti-GBSSI into the genome of potato plants

The plasmid pB33-anti-RL was constructed as described in Example 7. The plasmid pB33-anti-GBSSI was constructed as follows:
The DraI/DraI fragment of the promoter region of the patatin class I gene B33 from Solanum tuberosum comprising the nucleotides -1512 to +14 (Rocha-Sosa et al., EMBO J 8 (1989), 23-29) was ligated into the SmaI site of the pUC19 plasmid. From the resulting plasmid the promoter fragment was ligated into the polylinker region of the pBin19 plasmid (Bevan, Nucleic Acids Research 12 (1984), 8711-8721) as an EcoRI/HindIII fragment. Subsequently, the 3' EcoRI fragment 1181 to 2511 of the GBSSI gene of Solanum tuberosum (Hegersberg, dissertation (1988), University of Cologne) was ligated into the EcoRI site of the resulting plasmid.

Both plasmids were transferred sequentially into potato plants by means of Agrobacterium mediated transformation as described in Example 10. From the transformed cells plants were regenerated and the transformed plants were cultivated under greenhouse conditions. By analyzing the complete RNA in a RNA Blot analysis with regard to the disappearance of the transcripts complementary to the two cDNAs, the success of the genetic modification of the plants was assessed. For this

purpose, total RNA was isolated from tubers of transformed plants according to standard methods and subsequently separated on agarose gel by means of gel electrophoresis, transferred onto a membrane and hybridized with a radioactively labelled probe showing the sequence indicated under Seq ID No. 1 or a part thereof. Afterwards, the same membrane was hybridized with a radioactively labelled probe having the sequence of the GBSSI gene or a part of this sequence (Hegersberg, dissertation (1988) University of Cologne). In about 5-10% of the transformed plants the band indicating the transcripts hybridizing to the cDNA of the invention or the GBSSI cDNA were missing in the RNA Blot Analysis. From the tubers of these plants, which were designated R3 plants, starch was isolated and analyzed.

Example 12

Starch analysis of R4 plants

The potato plants transformed according to Example 10 were examined with respect to the properties of the synthesized starch. The analyses were carried out with various lines of the potato plants which had been transformed with the plasmids p35S-anti-RL and p35SH-anti-BE and which did no longer - or only in extremely reduced form - show the bands indicating transcripts hybridizing to the DNA sequences of the invention or to the sequences of the branching cDNA in RNA Blot analysis.

a) Determination of the viscosity of watery solutions of the starch

In order to determine the viscosity of the watery solutions of the starch synthesized in transformed potato

plants, starch was isolated from tubers of plants which had been transformed with the plasmid p35S-anti-RL and the plasmid p35SH-anti-BE. 2 g of starch were each dissolved in 25 ml H_2O and used for analysis with a Rapid Visco Analyser (Newport Scientific Pty Ltd, Investment Support Group, Warriewood NSW 2102, Australia). The equipment was used according to the instructions of the manufacturer. In order to determine the viscosity of the watery solution of the starch, the starch suspension was first heated from 50°C to 95°C with a speed of 12°C per minute. The temperature was then kept at 95°C for 2.5 minutes. Afterwards, the solution was cooled from 95°C to 50°C with a speed of 12°C per minute. During the whole process the viscosity was measured. Representative results of such measurements are set forth in the form of graphs in which the viscosity is shown depending on time. Figure 6 shows a typical RVA graph for starch isolated from the wildtype-plants of potato of the variety Désirée. Lines 2 and 3 show a typical RVA graph for starch isolated from the tubers of plants which had been transformed with the plasmid p35SH-anti-BE and with the plasmid p35S-anti-RL, respectively. Line 4 shows a typical RVA graph for starch isolated from tubers of plants which had been transformed with plasmid p35SH-anti-BE in combination with plasmid p35S-anti-RL. Line 4 is characterized in that there is no temperature-dependent increase of viscosity.

b) Determination of the amylose/amylopectin ratio

Starch which was isolated from the tubers of transformed potato plants was examined with respect to the ratio of amylose to amylopectin. The plant line R4-1 (shown in line 4 of Fig. 6) exhibited an amylose content of more than

70%. For the plant line R4-3 an amylose value of 27% was measured, whereas the amylose content in wildtype starch of the Désirée variety rates between 19 and 22%.

Example 13

Starch analysis of R3 plants

The potato plants transformed according to Example 11 were examined with respect to the properties of the synthesized starch. The analyses were carried out with various lines of the potato plants which had been transformed with the plasmids pB33-anti-RL and pB33-anti-GBSSI and which did no longer - or only in extremely reduced form - show the bands indicating transcripts hybridizing to the DNA sequences of the invention or to the sequences of the GBSSI cDNA in RNA Blot analysis.

a) Determination of the viscosity of watery solutions of the starch

In order to determine the viscosity of the watery solution of the starch synthesized in transformed potato plants, starch was isolated from tubers of plants which had been transformed with the plasmid pB33-anti-RL in combination with the plasmid pB33-anti-GBSSI. The viscosity was determined by means of a Rapid Visco Analyser according to the method described in Example 12, part a. The results are indicated in Figure 7. In line 1, Figure 7 shows a typical RVA graph for starch isolated from the wildtype-plants of the Désirée potato variety. Lines 2 and 3 show typical RVA graphs for starches isolated from potato plants which had been transformed with the plasmid pB33-anti-GBSSI and with the plasmid p35S-anti-RL,

respectively. Line 4 shows a typical RVA graph for starch isolated from potato plants which had been transformed with the plasmid pB33-anti-GBSSI in combination with the plasmid pB33-anti-RL. This graph is characterized in that the maximum viscosity and the increase of viscosity at 50°C are missing. Furthermore, this starch is characterized in that the glue obtained after RVA treatment exhibits almost no retrogradation after incubating at room temperature for several days.

b) Determination of the amylose/amylopectin ratio

Starch which was isolated from the tubers of transformed potato plants was examined with respect to the ratio of amylose to amylopectin. The plant line R3-5 (shown in line 4 of Fig. 7) exhibited an amylose content of less than 4%. For the plant line R3-6 an amylose content of less than 3% was measured. The amylose content in wildtype starch of the Désirée variety rates between 19 and 22%.

c) Determination of the phosphate content of starch

The phosphate content of the starch was determined by measuring the amount of phosphate bound to the C-6-position of the glucose residues. For this purpose, starch was first degraded by acid hydrolysis and the glucose-6-phosphate content was subsequently determined by means of an enzyme test, as described in the following.

100 mg starch were incubated in 500 µl 0.7 N HCl for 4 hours at 100°C. After acid hydrolysis 10 µl of the reaction mixture were added to 600 µl imidazole buffer (100 mM imidazole, 5 mM $MgCl_2$, pH 6.9, 0.4 mM NAD^+). The amount of glucose-6-phosphate in the preparation is

determined by conversion with the enzyme glucose-6-phosphate-dehydrogenase. For this purpose, 1 U glucose-6-phosphate-dehydrogenase (from Leuconostoc mesenteroides (Boehringer Mannheim)) was added to the reaction mixture and the amount of produced NADH was determined by measuring the absorption at 340 nm.

The glucose-6-phosphate content of 1 mg starch is indicated in the following table for non-transformed potato plants of the variety Désirée as well as for the R3-5 and the R3-6 line of transgenic potato plants which had been transformed with the plasmid pB33-anti-RL in combination with the plasmid pB33-anti-GBSSI. As a comparison, the value of the starch from the so-called waxy potato (US2-10) which had been transformed with the plasmid pB33-anti-GBSSI, is also indicated.

Table 7

Plants	nmol glucose-6-phosphate/mg starch	%
Wildtype	9.80 ± 0.68	100
R3-5	1.32 ± 0.10	13
R3-6	1.37 ± 0.15	14
US2-10	10.82 ± 0.42	110

Example 14

Isolation of a cDNA sequence encoding an R1 enzyme from Zea mays

Bacteria of the XL1-Blue strain were infected with lambda phages, the phage heads of which contained a cDNA library of endosperm tissue from Zea mays (Stratagene, Heidelberg). The infected E.coli cells were plated on a medium in Petri dishes

with a density of about 25000 plaques per approx. 75 cm^2. After about 9 hours of incubation nitro cellulose filters were laid on the lysed bacteria and were removed after one minute. The filter was first incubated in 0.5 M NaOH, 1.5 M NaCl for two minutes, then in 0.5 M Tris HCl pH 7.0 for two minutes and subsequently washed in 2x SSC for two minutes. After drying and fixing by UV crosslinking the filters were incubated in buffer A for 3 hours before a radioactively labelled DNA probe (random priming) was added. A fragment of the pRL2 plasmid DNA insertion (see Examples 4 and 5) with a size of approximately 2.7 was used as a probe. This fragment was cut with the restriction enzymes XhoI and HindIII and represented the 3' end of the cDNA insertion in pRL2 (see Figure 8).

After hybridizing for 12 hours at 48°C the filters were washed for 1 x 10 minutes in 2x SSC/1 % SDS at room temperature and then 2 x 20 minutes in 1 x SSC/0.5 % SDS at 35°C and subsequently autoradiographed.

Phage clones comprising a cDNA insertion were singled out in three screening cycles. Thereby, when screening about 1,500,000 phage plaques approximately 6 plaques were identified.

These positive phage clones were used for the in vivo excision of a pBluescript plasmid according to standard methods. The DNA sequences of the corresponding insertions were determined according to the method of Sanger et al. (Proc. Natl. Acad. Sci. USA 74 (1977), 5463-5467). Thus, a number of clones could be identified containing insertions encoding an R1 enzyme from maize. The cDNA insertion of a suitable clone, R1M, was completely determined. The nucleic acid sequence is indicated in Seq ID No. 5. The amino acid sequence derived therefrom is indicated in Seq ID No. 6.

A suitable cDNA insertion of the R1M clone was isolated from the pBluescript derivative by NotI and XhoI by means of standard methods (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbour Laboratory

Press, (1989), NY, USA). The sticky ends were filled in and the fragment was inserted into the pUBIbar vector at the HpaI site. This plasmid may be used for transforming plant cells, particularly maize, according to the methods described above. Since the sequence depicted in Seq ID No. 5 represents only a partial cDNA sequence, further techniques were applied to isolate sequences representing the 5' end of the cDNA. For this purpose polyA$^+$ RNA was isolated from leaf tissue of maize according to standard methods. The isolated RNA was used for a polymerase chain reaction using the Titan™ One Tube RT-PCR system (Boehringer Mannheim, Germany) according to the instructions of the manufacturer. In this reaction the RNA is transcribed in a first step into cDNA which is then used as a template for the PCR. As primers the following oligonuleotides were used:

Primer 1 (Seq ID No. 9):
5' GCAAAGTTTT CAAGGACAAG ACTGATGAAG 3'
Primer 2 (Seq ID No. 10):
5' CCAGATGGCA CGACAGTGTA CAAGAACA 3'
and
Primer 6 (Seq ID No. 11):
5' AATGACTGCA AAGGIGGIAT GATGGA 3'

The combination of primers 1 and 6 led to a 560 bp fragment. The primer combination 1 and 2 led to a PCR fragment of 2289 bp. Both fragments were sequenced. The obtained sequence represents most of the 5' end of the cDNA. The complete sequence of the partial cDNA clone and the sequences obtained by PCR as described above is depicted in Seq ID No. 7. The derived amino acid sequence is depicted in Seq ID No. 8.
Comparison with the full-length cDNA of potato revealed that the obtained sequence is probably not yet complete and that about 420 bp of the 5'end are missing. This missing sequence

can be completed by methods well known to the person skilled in the art. It is, for example, possible to isolate the 5'end of the cDNA using the 5'-RACE method (rapid amplification of cDNA ends). With this method an unknown 5'-end of a cDNA can be amplified by PCR. This method is normally used to produce cDNA which, in comparison to a known cDNA, is extended at the 5'-end. In order to apply the 5'-RACE method one can use, e.g., the Marathon-cDNA amplification kit (Clontech).
Other possibilities to isolate the complete cDNA are further PCRs using, for example, a lambda ZAP cDNA library of maize (Stratagene), immuno screening of expression libraries or the use of standard hybridization methods.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PlantTec Biotechnologie GmbH Forschung&Entwicklung
(B) STREET ADDRESS: Hermannswerder 14
(C) CITY: Potsdam
(E) COUNTRY: DE
(F) POSTAL CODE: 14473

(ii) TITLE OF INVENTION: Novel nucleic acid molecules from maize and their use for the production of modified starch

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER-READABLE VERSION:
(A) DATA CARRIER: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPA)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4856 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Solanum tuberosum
(B) STRAIN: C.V. Berolina

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:105..4497

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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CATCTTCATC GAATTTCTCG AAGCTTCTTC GCTAATTTCC TGGTTTCTTC ACTCAAAATC        60

GACGTTTCTA GCTGAACTTG AGTGAATTAA GCCAGTGGGA GGAT ATG AGT AAT TCC        116
                                                 Met Ser Asn Ser
                                                   1

TTA GGG AAT AAC TTG CTG TAC CAG GGA TTC CTA ACC TCA ACA GTG TTG         164
Leu Gly Asn Asn Leu Leu Tyr Gln Gly Phe Leu Thr Ser Thr Val Leu
  5                  10                  15                  20

GAA CAT AAA AGT AGA ATC AGT CCT CCT TGT GTT GGA GGC AAT TCT TTG         212
Glu His Lys Ser Arg Ile Ser Pro Pro Cys Val Gly Gly Asn Ser Leu
                 25                  30                  35
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TTT CAA CAA CAA GTG ATC TCG AAA TCA CCT TTA TCA ACT GAG TTT CGA 260
Phe Gln Gln Gln Val Ile Ser Lys Ser Pro Leu Ser Thr Glu Phe Arg
40 45 50

GGT AAC AGG TTA AAG GTG CAG AAA AAG AAA ATA CCT ATG GAA AAG AAG 308
Gly Asn Arg Leu Lys Val Gln Lys Lys Lys Ile Pro Met Glu Lys Lys
55 60 65

CGT GCT TTT TCT AGT TCT CCT CAT GCT GTA CTT ACC ACT GAT ACC TCT 356
Arg Ala Phe Ser Ser Ser Pro His Ala Val Leu Thr Thr Asp Thr Ser
70 75 80

TCT GAG CTA GCA GAA AAG TTC AGT CTA GGG GGG AAT ATT GAG CTA CAG 404
Ser Glu Leu Ala Glu Lys Phe Ser Leu Gly Gly Asn Ile Glu Leu Gln
85 90 95 100

GTT GAT GTT AGG CCT CCC ACT TCA GGT GAT GTG TCC TTT GTG GAT TTT 452
Val Asp Val Arg Pro Pro Thr Ser Gly Asp Val Ser Phe Val Asp Phe
105 110 115

CAA GTA ACA AAT GGT AGT GAT AAA CTG TTT TTG CAC TGG GGG GCA GTA 500
Gln Val Thr Asn Gly Ser Asp Lys Leu Phe Leu His Trp Gly Ala Val
120 125 130

AAA TTC GGG AAA GAA ACA TGG TCT CTT CCG AAT GAT CGT CCA GAT GGG 548
Lys Phe Gly Lys Glu Thr Trp Ser Leu Pro Asn Asp Arg Pro Asp Gly
135 140 145

ACC AAA GTG TAC AAG AAC AAA GCA CTT AGA ACT CCA TTT GTT AAA TCT 596
Thr Lys Val Tyr Lys Asn Lys Ala Leu Arg Thr Pro Phe Val Lys Ser
150 155 160

GGC TCT AAC TCC ATC CTG AGA CTG GAG ATA CGA GAC ACT GCT ATC GAA 644
Gly Ser Asn Ser Ile Leu Arg Leu Glu Ile Arg Asp Thr Ala Ile Glu
165 170 175 180

GCT ATT GAG TTT CTC ATA TAC GAT GAA GCC CAC GAT AAA TGG ATA AAG 692
Ala Ile Glu Phe Leu Ile Tyr Asp Glu Ala His Asp Lys Trp Ile Lys
185 190 195

AAT AAT GGT GGT AAT TTT CGT GTC AAA TTG TCA AGA AAA GAG ATA CGA 740
Asn Asn Gly Gly Asn Phe Arg Val Lys Leu Ser Arg Lys Glu Ile Arg
200 205 210

GGC CCA GAT GTT TCT GTT CCT GAG GAG CTT GTA CAG ATC CAA TCA TAT 788
Gly Pro Asp Val Ser Val Pro Glu Glu Leu Val Gln Ile Gln Ser Tyr
215 220 225

TTG AGG TGG GAG AGG AAG GGA AAA CAG AAT TAC CCC CCT GAG AAA GAG 836
Leu Arg Trp Glu Arg Lys Gly Lys Gln Asn Tyr Pro Pro Glu Lys Glu
230 235 240

AAG GAG GAA TAT GAG GCT GCT CGA ACT GTG CTA CAG GAG GAA ATA GCT 884
Lys Glu Glu Tyr Glu Ala Ala Arg Thr Val Leu Gln Glu Glu Ile Ala
245 250 255 260

CGT GGT GCT TCC ATA CAG GAC ATT CGA GCA AGG CTA ACA AAA ACT AAT 932
Arg Gly Ala Ser Ile Gln Asp Ile Arg Ala Arg Leu Thr Lys Thr Asn
265 270 275

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GAT AAA AGT CAA AGC AAA GAA GAG CCT CTT CAT GTA ACA AAG AGT GAT       980
Asp Lys Ser Gln Ser Lys Glu Glu Pro Leu His Val Thr Lys Ser Asp
            280                 285                 290

ATA CCT GAT GAC CTT GCC CAA GCA CAA GCT TAC ATT AGG TGG GAG AAA      1028
Ile Pro Asp Asp Leu Ala Gln Ala Gln Ala Tyr Ile Arg Trp Glu Lys
        295                 300                 305

GCA GGA AAG CCG AAC TAT CCT CCA GAA AAG CAA ATT GAA GAA CTC GAA      1076
Ala Gly Lys Pro Asn Tyr Pro Pro Glu Lys Gln Ile Glu Glu Leu Glu
    310                 315                 320

GAA GCA AGA AGA GAA TTG CAA CTT GAG CTT GAG AAA GGC ATT ACC CTT      1124
Glu Ala Arg Arg Glu Leu Gln Leu Glu Leu Glu Lys Gly Ile Thr Leu
325                 330                 335                 340

GAT GAG TTG CGG AAA ACG ATT ACA AAA GGG GAG ATA AAA ACT AAG GTG      1172
Asp Glu Leu Arg Lys Thr Ile Thr Lys Gly Glu Ile Lys Thr Lys Val
                345                 350                 355

GAA AAG CAC CTG AAA AGA AGT TCT TTT GCC GTT GAA AGA ATC CAA AGA      1220
Glu Lys His Leu Lys Arg Ser Ser Phe Ala Val Glu Arg Ile Gln Arg
            360                 365                 370

AAG AAG AGA GAC TTT GGG CAT CTT ATT AAT AAG TAT ACT TCC AGT CCT      1268
Lys Lys Arg Asp Phe Gly His Leu Ile Asn Lys Tyr Thr Ser Ser Pro
        375                 380                 385

GCA GTA CAA GTA CAA AAG GTC TTG GAA GAA CCA CCA GCC TTA TCT AAA      1316
Ala Val Gln Val Gln Lys Val Leu Glu Glu Pro Pro Ala Leu Ser Lys
    390                 395                 400

ATT AAG CTG TAT GCC AAG GAG AAG GAG GAG CAG ATT GAT GAT CCG ATC      1364
Ile Lys Leu Tyr Ala Lys Glu Lys Glu Glu Gln Ile Asp Asp Pro Ile
405                 410                 415                 420

CTA AAT AAA AAG ATC TTT AAG GTC GAT GAT GGG GAG CTA CTG GTA CTG      1412
Leu Asn Lys Lys Ile Phe Lys Val Asp Asp Gly Glu Leu Leu Val Leu
                425                 430                 435

GTA GCA AAG TCC TCT GGG AAG ACA AAA GTA CAT CTA GCT ACA GAT CTG      1460
Val Ala Lys Ser Ser Gly Lys Thr Lys Val His Leu Ala Thr Asp Leu
            440                 445                 450

AAT CAG CCA ATT ACT CTT CAC TGG GCA TTA TCC AAA AGT CCT GGA GAG      1508
Asn Gln Pro Ile Thr Leu His Trp Ala Leu Ser Lys Ser Pro Gly Glu
        455                 460                 465

TGG ATG GTA CCA CCT TCA AGC ATA TTG CCT CCT GGG TCA ATT ATT TTA      1556
Trp Met Val Pro Pro Ser Ser Ile Leu Pro Pro Gly Ser Ile Ile Leu
    470                 475                 480

GAC AAG GCT GCC GAA ACA CCT TTT TCA GCC AGT TCT TCT GAT GGT CTA      1604
Asp Lys Ala Ala Glu Thr Pro Phe Ser Ala Ser Ser Ser Asp Gly Leu
485                 490                 495                 500

ACT TCT AAG GTA CAA TCT TTG GAT ATA GTA ATT GAA GAT GGC AAT TTT      1652
Thr Ser Lys Val Gln Ser Leu Asp Ile Val Ile Glu Asp Gly Asn Phe
                505                 510                 515
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GTG GGG ATG CCA TTT GTT CTT TTG TCT GGT GAA AAA TGG ATT AAG AAC      1700
Val Gly Met Pro Phe Val Leu Leu Ser Gly Glu Lys Trp Ile Lys Asn
            520                 525                 530

CAA GGG TCG GAT TTC TAT GTT GGC TTC AGT GCT GCA TCC AAA TTA GCA      1748
Gln Gly Ser Asp Phe Tyr Val Gly Phe Ser Ala Ala Ser Lys Leu Ala
        535                 540                 545

CTC AAG GCT GCT GGG GAT GGC AGT GGA ACT GCA AAG TCT TTA CTG GAT      1796
Leu Lys Ala Ala Gly Asp Gly Ser Gly Thr Ala Lys Ser Leu Leu Asp
    550                 555                 560

AAA ATA GCA GAT ATG GAA AGT GAG GCT CAG AAG TCA TTT ATG CAC CGG      1844
Lys Ile Ala Asp Met Glu Ser Glu Ala Gln Lys Ser Phe Met His Arg
565                 570                 575                 580

TTT AAT ATT GCA GCT GAC TTG ATA GAA GAT GCC ACT AGT GCT GGT GAA      1892
Phe Asn Ile Ala Ala Asp Leu Ile Glu Asp Ala Thr Ser Ala Gly Glu
                585                 590                 595

CTT GGT TTT GCT GGA ATT CTT GTA TGG ATG AGG TTC ATG GCT ACA AGG      1940
Leu Gly Phe Ala Gly Ile Leu Val Trp Met Arg Phe Met Ala Thr Arg
            600                 605                 610

CAA CTG ATA TGG AAC AAA AAC TAT AAC GTA AAA CCA CGT GAA ATA AGC      1988
Gln Leu Ile Trp Asn Lys Asn Tyr Asn Val Lys Pro Arg Glu Ile Ser
        615                 620                 625

AAG GCT CAG GAC AGA CTT ACA GAC TTG TTG CAG AAT GCT TTC ACC AGT      2036
Lys Ala Gln Asp Arg Leu Thr Asp Leu Leu Gln Asn Ala Phe Thr Ser
    630                 635                 640

CAC CCT CAG TAC CGT GAA ATT TTG CGG ATG ATT ATG TCA ACT GTT GGA      2084
His Pro Gln Tyr Arg Glu Ile Leu Arg Met Ile Met Ser Thr Val Gly
645                 650                 655                 660

CGT GGA GGT GAA GGG GAT GTA GGA CAG CGA ATT AGG GAT GAA ATT TTG      2132
Arg Gly Gly Glu Gly Asp Val Gly Gln Arg Ile Arg Asp Glu Ile Leu
                665                 670                 675

GTC ATC CAG AGG AAC AAT GAC TGC AAG GGT GGT ATG ATG CAA GAA TGG      2180
Val Ile Gln Arg Asn Asn Asp Cys Lys Gly Gly Met Met Gln Glu Trp
            680                 685                 690

CAT CAG AAA TTG CAT AAT AAT ACT AGT CCT GAT GAT GTT GTG ATC TGT      2228
His Gln Lys Leu His Asn Asn Thr Ser Pro Asp Asp Val Val Ile Cys
        695                 700                 705

CAG GCA TTA ATT GAC TAC ATC AAG AGT GAT TTT GAT CTT GGT GTT TAT      2276
Gln Ala Leu Ile Asp Tyr Ile Lys Ser Asp Phe Asp Leu Gly Val Tyr
    710                 715                 720

TGG AAA ACC CTG AAT GAG AAC GGA ATA ACA AAA GAG CGT CTT TTG AGT      2324
Trp Lys Thr Leu Asn Glu Asn Gly Ile Thr Lys Glu Arg Leu Leu Ser
725                 730                 735                 740
TAT GAC CGT GCT ATC CAT TCT GAA CCA AAT TTT AGA GGA GAT CAA AAG      2372
Tyr Asp Arg Ala Ile His Ser Glu Pro Asn Phe Arg Gly Asp Gln Lys
                745                 750                 755
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GGT GGT CTT TTG CGT GAT TTA GGT CAC TAT ATG AGA ACA TTG AAG GCA        2420
Gly Gly Leu Leu Arg Asp Leu Gly His Tyr Met Arg Thr Leu Lys Ala
        760                 765                 770

GTT CAT TCA GGT GCA GAT CTT GAG TCT GCT ATT GCA AAC TGC ATG GGC        2468
Val His Ser Gly Ala Asp Leu Glu Ser Ala Ile Ala Asn Cys Met Gly
    775                 780                 785

TAC AAA ACT GAG GGA GAA GGC TTT ATG GTT GGA GTC CAG ATA AAT CCT        2516
Tyr Lys Thr Glu Gly Glu Gly Phe Met Val Gly Val Gln Ile Asn Pro
    790                 795                 800

GTA TCA GGC TTG CCA TCT GGC TTT CAG GAC CTC CTC CAT TTT GTC TTA        2564
Val Ser Gly Leu Pro Ser Gly Phe Gln Asp Leu Leu His Phe Val Leu
805                 810                 815                 820

GAC CAT GTG GAA GAT AAA AAT GTG GAA ACT CTT CTT GAG AGA TTG CTA        2612
Asp His Val Glu Asp Lys Asn Val Glu Thr Leu Leu Glu Arg Leu Leu
                825                 830                 835

GAG GCT CGT GAG GAG CTT AGG CCC TTG CTT CTC AAA CCA AAC AAC CGT        2660
Glu Ala Arg Glu Glu Leu Arg Pro Leu Leu Leu Lys Pro Asn Asn Arg
            840                 845                 850

CTA AAG GAT CTG CTG TTT TTG GAC ATA GCA CTT GAT TCT ACA GTT AGA        2708
Leu Lys Asp Leu Leu Phe Leu Asp Ile Ala Leu Asp Ser Thr Val Arg
        855                 860                 865

ACA GCA GTA GAA AGG GGA TAT GAA GAA TTG AAC AAC GCT AAT CCT GAG        2756
Thr Ala Val Glu Arg Gly Tyr Glu Glu Leu Asn Asn Ala Asn Pro Glu
    870                 875                 880

AAA ATC ATG TAC TTC ATC TCC CTC GTT CTT GAA AAT CTC GCA CTC TCT        2804
Lys Ile Met Tyr Phe Ile Ser Leu Val Leu Glu Asn Leu Ala Leu Ser
885                 890                 895                 900

GTG GAC GAT AAT GAA GAT CTT GTT TAT TGC TTG AAG GGA TGG AAT CAA        2852
Val Asp Asp Asn Glu Asp Leu Val Tyr Cys Leu Lys Gly Trp Asn Gln
                905                 910                 915

GCT CTT TCA ATG TCC AAT GGT GGG GAC AAC CAT TGG GCT TTA TTT GCA        2900
Ala Leu Ser Met Ser Asn Gly Gly Asp Asn His Trp Ala Leu Phe Ala
            920                 925                 930

AAA GCT GTG CTT GAC AGA ACC CGT CTT GCA CTT GCA AGC AAG GCA GAG        2948
Lys Ala Val Leu Asp Arg Thr Arg Leu Ala Leu Ala Ser Lys Ala Glu
        935                 940                 945

TGG TAC CAT CAC TTA TTG CAG CCA TCT GCC GAA TAT CTA GGA TCA ATA        2996
Trp Tyr His His Leu Leu Gln Pro Ser Ala Glu Tyr Leu Gly Ser Ile
    950                 955                 960

CTT GGG GTG GAC CAA TGG GCT TTG AAC ATA TTT ACT GAA GAA ATT ATA        3044
Leu Gly Val Asp Gln Trp Ala Leu Asn Ile Phe Thr Glu Glu Ile Ile
965                 970                 975                 980

CGT GCT GGA TCA GCA GCT TCA TTA TCC TCT CTT CTT AAT AGA CTC GAT        3092
Arg Ala Gly Ser Ala Ala Ser Leu Ser Ser Leu Leu Asn Arg Leu Asp
                985                 990                 995
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CCC GTG CTT CGG AAA ACT GCA AAT CTA GGA AGT TGG CAG ATT ATC AGT 3140
Pro Val Leu Arg Lys Thr Ala Asn Leu Gly Ser Trp Gln Ile Ile Ser
1000 1005 1010

CCA GTT GAA GCC GTT GGA TAT GTT GTC GTT GTG GAT GAG TTG CTT TCA 3188
Pro Val Glu Ala Val Gly Tyr Val Val Val Val Asp Glu Leu Leu Ser
1015 1020 1025

GTT CAG AAT GAA ATC TAC GAG AAG CCC ACG ATC TTA GTA GCA AAA TCT 3236
Val Gln Asn Glu Ile Tyr Glu Lys Pro Thr Ile Leu Val Ala Lys Ser
1030 1035 1040

GTT AAA GGA GAG GAG GAA ATT CCT GAT GGT GCT GTT GCC CTG ATA ACA 3284
Val Lys Gly Glu Glu Glu Ile Pro Asp Gly Ala Val Ala Leu Ile Thr
1045 1050 1055 1060

CCA GAC ATG CCA GAT GTT CTT TCA CAT GTT TCT GTT CGA GCT AGA AAT 3332
Pro Asp Met Pro Asp Val Leu Ser His Val Ser Val Arg Ala Arg Asn
1065 1070 1075

GGG AAG GTT TGC TTT GCT ACA TGC TTT GAT CCC AAT ATA TTG GCT GAC 3380
Gly Lys Val Cys Phe Ala Thr Cys Phe Asp Pro Asn Ile Leu Ala Asp
1080 1085 1090

CTC CAA GCA AAG GAA GGA AGG ATT TTG CTC TTA AAG CCT ACA CCT TCA 3428
Leu Gln Ala Lys Glu Gly Arg Ile Leu Leu Leu Lys Pro Thr Pro Ser
1095 1100 1105

GAC ATA ATC TAT AGT GAG GTG AAT GAG ATT GAG CTC CAA AGT TCA AGT 3476
Asp Ile Ile Tyr Ser Glu Val Asn Glu Ile Glu Leu Gln Ser Ser Ser
1110 1115 1120

AAC TTG GTA GAA GCT GAA ACT TCA GCA ACA CTT AGA TTG GTG AAA AAG 3524
Asn Leu Val Glu Ala Glu Thr Ser Ala Thr Leu Arg Leu Val Lys Lys
1125 1130 1135 1140

CAA TTT GGT GGT TGT TAC GCA ATA TCA GCA GAT GAA TTC ACA AGT GAA 3572
Gln Phe Gly Gly Cys Tyr Ala Ile Ser Ala Asp Glu Phe Thr Ser Glu
1145 1150 1155

ATG GTT GGA GCT AAA TCA CGT AAT ATT GCA TAT CTG AAA GGA AAA GTG 3620
Met Val Gly Ala Lys Ser Arg Asn Ile Ala Tyr Leu Lys Gly Lys Val
1160 1165 1170

CCT TCC TCG GTG GGA ATT CCT ACG TCA GTA GCT CTT CCA TTT GGA GTC 3668
Pro Ser Ser Val Gly Ile Pro Thr Ser Val Ala Leu Pro Phe Gly Val
1175 1180 1185

TTT GAG AAA GTA CTT TCA GAC GAC ATA AAT CAG GGA GTG GCA AAA GAG 3716
Phe Glu Lys Val Leu Ser Asp Asp Ile Asn Gln Gly Val Ala Lys Glu
1190 1195 1200

TTG CAA ATT CTG ATG AAA AAA CTA TCT GAA GGA GAC TTC AGC GCT CTT 3764
Leu Gln Ile Leu Met Lys Lys Leu Ser Glu Gly Asp Phe Ser Ala Leu
1205 1210 1215 1220

GGT GAA ATT CGC ACA ACG GTT TTA GAT CTT TCA GCA CCA GCT CAA TTG 3812
Gly Glu Ile Arg Thr Thr Val Leu Asp Leu Ser Ala Pro Ala Gln Leu
1225 1230 1235

GTC AAA GAG CTG AAG GAG AAG ATG CAG GGT TCT GGC ATG CCT TGG CCT 3860
Val Lys Glu Leu Lys Glu Lys Met Gln Gly Ser Gly Met Pro Trp Pro
1240 1245 1250

GGT GAT GAA GGT CCA AAG CGG TGG GAA CAA GCA TGG ATG GCC ATA AAA 3908
Gly Asp Glu Gly Pro Lys Arg Trp Glu Gln Ala Trp Met Ala Ile Lys
1255 1260 1265

AAG GTG TGG GCT TCA AAA TGG AAT GAG AGA GCA TAC TTC AGC ACA AGG 3956
Lys Val Trp Ala Ser Lys Trp Asn Glu Arg Ala Tyr Phe Ser Thr Arg
1270 1275 1280

AAG GTG AAA CTG GAT CAT GAC TAT CTG TGC ATG GCT GTC CTT GTT CAA 4004
Lys Val Lys Leu Asp His Asp Tyr Leu Cys Met Ala Val Leu Val Gln
1285 1290 1295 1300

GAA ATA ATA AAT GCT GAT TAT GCA TTT GTC ATT CAC ACA ACC AAC CCA 4052
Glu Ile Ile Asn Ala Asp Tyr Ala Phe Val Ile His Thr Thr Asn Pro
1305 1310 1315

TCT TCC GGA GAC GAC TCA GAA ATA TAT GCC GAG GTG GTC AGG GGC CTT 4100
Ser Ser Gly Asp Asp Ser Glu Ile Tyr Ala Glu Val Val Arg Gly Leu
1320 1325 1330

GGG GAA ACA CTT GTT GGA GCT TAT CCA GGA CGT GCT TTG AGT TTT ATC 4148
Gly Glu Thr Leu Val Gly Ala Tyr Pro Gly Arg Ala Leu Ser Phe Ile
1335 1340 1345

TGC AAG AAA AAG GAT CTC AAC TCT CCT CAA GTG TTA GGT TAC CCA AGC 4196
Cys Lys Lys Lys Asp Leu Asn Ser Pro Gln Val Leu Gly Tyr Pro Ser
1350 1355 1360

AAA CCG ATC GGC CTT TTC ATA AAA AGA TCT ATC ATC TTC CGA TCT GAT 4244
Lys Pro Ile Gly Leu Phe Ile Lys Arg Ser Ile Ile Phe Arg Ser Asp
1365 1370 1375 1380

TCC AAT GGG GAA GAT TTG GAA GGT TAT GCC GGT GCT GGC CTC TAC GAC 4292
Ser Asn Gly Glu Asp Leu Glu Gly Tyr Ala Gly Ala Gly Leu Tyr Asp
1385 1390 1395

AGT GTA CCA ATG GAT GAG GAG GAA AAA GTT GTA ATT GAT TAC TCT TCC 4340
Ser Val Pro Met Asp Glu Glu Glu Lys Val Val Ile Asp Tyr Ser Ser
1400 1405 1410

GAC CCA TTG ATA ACT GAT GGT AAC TTC CGC CAG ACA ATC CTG TCC AAC 4388
Asp Pro Leu Ile Thr Asp Gly Asn Phe Arg Gln Thr Ile Leu Ser Asn
1415 1420 1425

ATT GCT CGT GCT GGA CAT GCT ATC GAG GAG CTA TAT GGC TCT CCT CAA 4436
Ile Ala Arg Ala Gly His Ala Ile Glu Glu Leu Tyr Gly Ser Pro Gln
1430 1435 1440

GAC ATT GAG GGT GTA GTG AGG GAT GGA AAG ATT TAT GTC GTT CAG ACA 4484
Asp Ile Glu Gly Val Val Arg Asp Gly Lys Ile Tyr Val Val Gln Thr
1445 1450 1455 1460

AGA CCA CAG ATG T GATTATATTC TCGTTGTATG TTGTTCAGAG AAGACCACAG 4537
Arg Pro Gln Met

ATGTGATCAT ATTCTCATTG TATCAGATCT GTGACCACTT ACCTGATACC TCCCATGAAG 4597

TTACCTGTAT GATTATACGT GATCCAAAGC CATCACATCA TGTTCACCTT CAGCTATTGG 4657

AGGAGAAGTG AGAAGTAGGA ATTGCAATAT GAGGAATAAT AAGAAAAACT TTGTAAAAGC 4717

TAAATTAGCT GGGTATGATA TAGGGAGAAA TGTGTAAACA TTGTACTATA TATAGTATAT 4777

ACACACGCAT TATGTATTGC ATTATGCACT GAATAATATC GCAGCATCAA AGAAGAAATC 4837

CTTTGGGTGG TTTCAAAAA 4856

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1464 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ser Asn Ser Leu Gly Asn Asn Leu Leu Tyr Gln Gly Phe Leu Thr
1 5 10 15

Ser Thr Val Leu Glu His Lys Ser Arg Ile Ser Pro Pro Cys Val Gly
20 25 30

Gly Asn Ser Leu Phe Gln Gln Gln Val Ile Ser Lys Ser Pro Leu Ser
35 40 45

Thr Glu Phe Arg Gly Asn Arg Leu Lys Val Gln Lys Lys Lys Ile Pro
50 55 60

Met Glu Lys Lys Arg Ala Phe Ser Ser Ser Pro His Ala Val Leu Thr
65 70 75 80

Thr Asp Thr Ser Ser Glu Leu Ala Glu Lys Phe Ser Leu Gly Gly Asn
85 90 95

Ile Glu Leu Gln Val Asp Val Arg Pro Pro Thr Ser Gly Asp Val Ser
100 105 110

Phe Val Asp Phe Gln Val Thr Asn Gly Ser Asp Lys Leu Phe Leu His
115 120 125

Trp Gly Ala Val Lys Phe Gly Lys Glu Thr Trp Ser Leu Pro Asn Asp
130 135 140

Arg Pro Asp Gly Thr Lys Val Tyr Lys Asn Lys Ala Leu Arg Thr Pro
145 150 155 160

Phe Val Lys Ser Gly Ser Asn Ser Ile Leu Arg Leu Glu Ile Arg Asp
165 170 175

Thr Ala Ile Glu Ala Ile Glu Phe Leu Ile Tyr Asp Glu Ala His Asp
180 185 190

Lys Trp Ile Lys Asn Asn Gly Gly Asn Phe Arg Val Lys Leu Ser Arg
195 200 205

Lys Glu Ile Arg Gly Pro Asp Val Ser Val Pro Glu Glu Leu Val Gln
210 215 220

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Ile Gln Ser Tyr Leu Arg Trp Glu Arg Lys Gly Lys Gln Asn Tyr Pro
225                 230                 235                 240

Pro Glu Lys Glu Lys Glu Glu Tyr Glu Ala Ala Arg Thr Val Leu Gln
                245                 250                 255

Glu Glu Ile Ala Arg Gly Ala Ser Ile Gln Asp Ile Arg Ala Arg Leu
            260                 265                 270

Thr Lys Thr Asn Asp Lys Ser Gln Ser Lys Glu Glu Pro Leu His Val
        275                 280                 285

Thr Lys Ser Asp Ile Pro Asp Asp Leu Ala Gln Ala Gln Ala Tyr Ile
    290                 295                 300

Arg Trp Glu Lys Ala Gly Lys Pro Asn Tyr Pro Pro Glu Lys Gln Ile
305                 310                 315                 320

Glu Glu Leu Glu Glu Ala Arg Arg Glu Leu Gln Leu Glu Leu Glu Lys
                325                 330                 335

Gly Ile Thr Leu Asp Glu Leu Arg Lys Thr Ile Thr Lys Gly Glu Ile
            340                 345                 350

Lys Thr Lys Val Glu Lys His Leu Lys Arg Ser Ser Phe Ala Val Glu
        355                 360                 365

Arg Ile Gln Arg Lys Lys Arg Asp Phe Gly His Leu Ile Asn Lys Tyr
    370                 375                 380

Thr Ser Ser Pro Ala Val Gln Val Gln Lys Val Leu Glu Glu Pro Pro
385                 390                 395                 400

Ala Leu Ser Lys Ile Lys Leu Tyr Ala Lys Glu Lys Glu Glu Gln Ile
                405                 410                 415

Asp Asp Pro Ile Leu Asn Lys Lys Ile Phe Lys Val Asp Asp Gly Glu
            420                 425                 430

Leu Leu Val Leu Val Ala Lys Ser Ser Gly Lys Thr Lys Val His Leu
        435                 440                 445

Ala Thr Asp Leu Asn Gln Pro Ile Thr Leu His Trp Ala Leu Ser Lys
    450                 455                 460

Ser Pro Gly Glu Trp Met Val Pro Pro Ser Ser Ile Leu Pro Pro Gly
465                 470                 475                 480

Ser Ile Ile Leu Asp Lys Ala Ala Glu Thr Pro Phe Ser Ala Ser Ser
                485                 490                 495

Ser Asp Gly Leu Thr Ser Lys Val Gln Ser Leu Asp Ile Val Ile Glu
            500                 505                 510

Asp Gly Asn Phe Val Gly Met Pro Phe Val Leu Leu Ser Gly Glu Lys
        515                 520                 525

Trp Ile Lys Asn Gln Gly Ser Asp Phe Tyr Val Gly Phe Ser Ala Ala
    530                 535                 540
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Ser Lys Leu Ala Leu Lys Ala Ala Gly Asp Gly Ser Gly Thr Ala Lys
545   550   555   560

Ser Leu Leu Asp Lys Ile Ala Asp Met Glu Ser Glu Ala Gln Lys Ser
                565   570   575

Phe Met His Arg Phe Asn Ile Ala Ala Asp Leu Ile Glu Asp Ala Thr
            580   585   590

Ser Ala Gly Glu Leu Gly Phe Ala Gly Ile Leu Val Trp Met Arg Phe
        595   600   605

Met Ala Thr Arg Gln Leu Ile Trp Asn Lys Asn Tyr Asn Val Lys Pro
    610   615   620

Arg Glu Ile Ser Lys Ala Gln Asp Arg Leu Thr Asp Leu Leu Gln Asn
625   630   635   640

Ala Phe Thr Ser His Pro Gln Tyr Arg Glu Ile Leu Arg Met Ile Met
                645   650   655

Ser Thr Val Gly Arg Gly Gly Glu Gly Asp Val Gly Gln Arg Ile Arg
            660   665   670

Asp Glu Ile Leu Val Ile Gln Arg Asn Asn Asp Cys Lys Gly Gly Met
        675   680   685

Met Gln Glu Trp His Gln Lys Leu His Asn Asn Thr Ser Pro Asp Asp
    690   695   700

Val Val Ile Cys Gln Ala Leu Ile Asp Tyr Ile Lys Ser Asp Phe Asp
705   710   715   720

Leu Gly Val Tyr Trp Lys Thr Leu Asn Glu Asn Gly Ile Thr Lys Glu
                725   730   735

Arg Leu Leu Ser Tyr Asp Arg Ala Ile His Ser Glu Pro Asn Phe Arg
            740   745   750

Gly Asp Gln Lys Gly Gly Leu Leu Arg Asp Leu Gly His Tyr Met Arg
        755   760   765

Thr Leu Lys Ala Val His Ser Gly Ala Asp Leu Glu Ser Ala Ile Ala
    770   775   780

Asn Cys Met Gly Tyr Lys Thr Glu Gly Glu Gly Phe Met Val Gly Val
785   790   795   800

Gln Ile Asn Pro Val Ser Gly Leu Pro Ser Gly Phe Gln Asp Leu Leu
                805   810   815

His Phe Val Leu Asp His Val Glu Asp Lys Asn Val Glu Thr Leu Leu
            820   825   830

Glu Arg Leu Leu Glu Ala Arg Glu Glu Leu Arg Pro Leu Leu Leu Lys
        835   840   845

Pro Asn Asn Arg Leu Lys Asp Leu Leu Phe Leu Asp Ile Ala Leu Asp
    850   855   860

Ser Thr Val Arg Thr Ala Val Glu Arg Gly Tyr Glu Glu Leu Asn Asn
865   870   875   880

Ala Asn Pro Glu Lys Ile Met Tyr Phe Ile Ser Leu Val Leu Glu Asn
885 890 895

Leu Ala Leu Ser Val Asp Asp Asn Glu Asp Leu Val Tyr Cys Leu Lys
900 905 910

Gly Trp Asn Gln Ala Leu Ser Met Ser Asn Gly Gly Asp Asn His Trp
915 920 925

Ala Leu Phe Ala Lys Ala Val Leu Asp Arg Thr Arg Leu Ala Leu Ala
930 935 940

Ser Lys Ala Glu Trp Tyr His His Leu Leu Gln Pro Ser Ala Glu Tyr
945 950 955 960

Leu Gly Ser Ile Leu Gly Val Asp Gln Trp Ala Leu Asn Ile Phe Thr
965 970 975

Glu Glu Ile Ile Arg Ala Gly Ser Ala Ala Ser Leu Ser Ser Leu Leu
980 985 990

Asn Arg Leu Asp Pro Val Leu Arg Lys Thr Ala Asn Leu Gly Ser Trp
995 1000 1005

Gln Ile Ile Ser Pro Val Glu Ala Val Gly Tyr Val Val Val Val Asp
1010 1015 1020

Glu Leu Leu Ser Val Gln Asn Glu Ile Tyr Glu Lys Pro Thr Ile Leu
1025 1030 1035 1040

Val Ala Lys Ser Val Lys Gly Glu Glu Glu Ile Pro Asp Gly Ala Val
1045 1050 1055

Ala Leu Ile Thr Pro Asp Met Pro Asp Val Leu Ser His Val Ser Val
1060 1065 1070

Arg Ala Arg Asn Gly Lys Val Cys Phe Ala Thr Cys Phe Asp Pro Asn
1075 1080 1085

Ile Leu Ala Asp Leu Gln Ala Lys Glu Gly Arg Ile Leu Leu Leu Lys
1090 1095 1100

Pro Thr Pro Ser Asp Ile Ile Tyr Ser Glu Val Asn Glu Ile Glu Leu
1105 1110 1115 1120

Gln Ser Ser Ser Asn Leu Val Glu Ala Glu Thr Ser Ala Thr Leu Arg
1125 1130 1135

Leu Val Lys Lys Gln Phe Gly Gly Cys Tyr Ala Ile Ser Ala Asp Glu
1140 1145 1150

Phe Thr Ser Glu Met Val Gly Ala Lys Ser Arg Asn Ile Ala Tyr Leu
1155 1160 1165

Lys Gly Lys Val Pro Ser Ser Val Gly Ile Pro Thr Ser Val Ala Leu
1170 1175 1180

Pro Phe Gly Val Phe Glu Lys Val Leu Ser Asp Asp Ile Asn Gln Gly
1185 1190 1195 1200

Val Ala Lys Glu Leu Gln Ile Leu Met Lys Lys Leu Ser Glu Gly Asp
1205 1210 1215

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Phe Ser Ala Leu Gly Glu Ile Arg Thr Thr Val Leu Asp Leu Ser Ala
            1220                1225                1230

Pro Ala Gln Leu Val Lys Glu Leu Lys Glu Lys Met Gln Gly Ser Gly
        1235                1240                1245

Met Pro Trp Pro Gly Asp Glu Gly Pro Lys Arg Trp Glu Gln Ala Trp
    1250                1255                1260

Met Ala Ile Lys Lys Val Trp Ala Ser Lys Trp Asn Glu Arg Ala Tyr
1265                1270                1275                1280

Phe Ser Thr Arg Lys Val Lys Leu Asp His Asp Tyr Leu Cys Met Ala
                1285                1290                1295

Val Leu Val Gln Glu Ile Ile Asn Ala Asp Tyr Ala Phe Val Ile His
            1300                1305                1310

Thr Thr Asn Pro Ser Ser Gly Asp Asp Ser Glu Ile Tyr Ala Glu Val
        1315                1320                1325

Val Arg Gly Leu Gly Glu Thr Leu Val Gly Ala Tyr Pro Gly Arg Ala
    1330                1335                1340

Leu Ser Phe Ile Cys Lys Lys Lys Asp Leu Asn Ser Pro Gln Val Leu
1345                1350                1355                1360

Gly Tyr Pro Ser Lys Pro Ile Gly Leu Phe Ile Lys Arg Ser Ile Ile
                1365                1370                1375

Phe Arg Ser Asp Ser Asn Gly Glu Asp Leu Glu Gly Tyr Ala Gly Ala
            1380                1385                1390


Gly Leu Tyr Asp Ser Val Pro Met Asp Glu Glu Glu Lys Val Val Ile
        1395                1400                1405

Asp Tyr Ser Ser Asp Pro Leu Ile Thr Asp Gly Asn Phe Arg Gln Thr
    1410                1415                1420

Ile Leu Ser Asn Ile Ala Arg Ala Gly His Ala Ile Glu Glu Leu Tyr
1425                1430                1435                1440

Gly Ser Pro Gln Asp Ile Glu Gly Val Val Arg Asp Gly Lys Ile Tyr
                1445                1450                1455
Val Val Gln Thr Arg Pro Gln Met
            1460
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1918 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:
- (A) ORGANISM: Solanum tuberosum

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          (B) STRAIN: C.V. Desiree

    (ix) FEATURE:
          (A) NAME/KEY: CDS
          (B) LOCATION:1..1555

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCA GAG TGG TAC CAT CAC TTA TTG CAG CCA TCT GCC GAA TAT CTA GGA        48
Ala Glu Trp Tyr His His Leu Leu Gln Pro Ser Ala Glu Tyr Leu Gly
  1               5                  10                  15

TCA ATA CTT GGG GTG GAC CAA TGG GCT TTG AAC ATA TTT ACT GAA GAA        96
Ser Ile Leu Gly Val Asp Gln Trp Ala Leu Asn Ile Phe Thr Glu Glu
             20                  25                  30

ATT ATA CGT GCT GGA TCA GCA GCT TCA TTA TCC TCT CTT CTT AAT AGA       144
Ile Ile Arg Ala Gly Ser Ala Ala Ser Leu Ser Ser Leu Leu Asn Arg
         35                  40                  45

CTC GAT CCC GTG CTT CGG AAA ACT GCA AAT CTA GGA AGT TGG CAG ATT       192
Leu Asp Pro Val Leu Arg Lys Thr Ala Asn Leu Gly Ser Trp Gln Ile
     50                  55                  60

ATC AGT CCA GTT GAA GCC GTT GGA TAT GTT GTC GTT GTG GAT GAG TTG       240
Ile Ser Pro Val Glu Ala Val Gly Tyr Val Val Val Val Asp Glu Leu
 65                  70                  75                  80

CTT TCA GTT CAG AAT GAA ATC TAC GAG AAG CCC ACG ATC TTA GTA GCA       288
Leu Ser Val Gln Asn Glu Ile Tyr Glu Lys Pro Thr Ile Leu Val Ala
                 85                  90                  95

AAA TCT GTT AAA GGA GAG GAG GAA ATT CCT GAT GGT GCT GTT GCC CTG       336
Lys Ser Val Lys Gly Glu Glu Glu Ile Pro Asp Gly Ala Val Ala Leu
            100                 105                 110

ATA ACA CCA GAC ATG CCA GAT GTT CTT TCA CAT GTT TCT GTT CGA GCT       384
Ile Thr Pro Asp Met Pro Asp Val Leu Ser His Val Ser Val Arg Ala
        115                 120                 125

AGA AAT GGG AAG GTT TGC TTT GCT ACA TGC TTT GAT CCC AAT ATA TTG       432
Arg Asn Gly Lys Val Cys Phe Ala Thr Cys Phe Asp Pro Asn Ile Leu
    130                 135                 140
GCT GAC CTC CAA GCA AAG GAA GGA AGG ATT TTG CTC TTA AAG CCT ACA       480
Ala Asp Leu Gln Ala Lys Glu Gly Arg Ile Leu Leu Leu Lys Pro Thr
145                 150                 155                 160

CCT TCA GAC ATA ATC TAT AGT GAG GTG AAT GAG ATT GAG CTC CAA AGT       528
Pro Ser Asp Ile Ile Tyr Ser Glu Val Asn Glu Ile Glu Leu Gln Ser
                165                 170                 175

TCA AGT AAC TTG GTA GAA GCT GAA ACT TCA GCA ACA CTT AGA TTG GTG       576
Ser Ser Asn Leu Val Glu Ala Glu Thr Ser Ala Thr Leu Arg Leu Val
            180                 185                 190

AAA AAG CAA TTT GGT GGT TGT TAC GCA ATA TCA GCA GAT GAA TTC ACA       624
Lys Lys Gln Phe Gly Gly Cys Tyr Ala Ile Ser Ala Asp Glu Phe Thr
        195                 200                 205
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AGT GAA ATG GTT GGA GCT AAA TCA CGT AAT ATT GCA TAT CTG AAA GGA      672
Ser Glu Met Val Gly Ala Lys Ser Arg Asn Ile Ala Tyr Leu Lys Gly
    210                 215                 220

AAA GTG CCT TCC TCG GTG GGA ATT CCT ACG TCA GTA GCT CTT CCA TTT      720
Lys Val Pro Ser Ser Val Gly Ile Pro Thr Ser Val Ala Leu Pro Phe
225                 230                 235                 240

GGA GTC TTT GAG AAA GTA CTT TCA GAC GAC ATA AAT CAG GGA GTG GCA      768
Gly Val Phe Glu Lys Val Leu Ser Asp Asp Ile Asn Gln Gly Val Ala
                245                 250                 255

AAA GAG TTG CAA ATT CTG ACA AAA AAA CTA TCT GAA GGA GAC TTT AGC      816
Lys Glu Leu Gln Ile Leu Thr Lys Lys Leu Ser Glu Gly Asp Phe Ser
            260                 265                 270

GCT CTT GGT GAA ATT CGC ACA ACG GTT TTA GAT CTT TCG ACA CCA GCT      864
Ala Leu Gly Glu Ile Arg Thr Thr Val Leu Asp Leu Ser Thr Pro Ala
        275                 280                 285

CAA TTG GTC AAA GAG CTG AAG GAG AAG ATG CAG GGT TCT GGC ATG CCT      912
Gln Leu Val Lys Glu Leu Lys Glu Lys Met Gln Gly Ser Gly Met Pro
    290                 295                 300

TGG CCT GGT GAT GAA GGT CCA AAG CGG TGG GAA CAA GCA TGG ATG GCC      960
Trp Pro Gly Asp Glu Gly Pro Lys Arg Trp Glu Gln Ala Trp Met Ala
305                 310                 315                 320

ATA AAA AAG GTG TGG GCT TCA AAA TGG AAT GAG AGA GCA TAC TTC AGC     1008
Ile Lys Lys Val Trp Ala Ser Lys Trp Asn Glu Arg Ala Tyr Phe Ser
                325                 330                 335

ACA AGG AAG GTG AAA CTG GAT CAT GAC TAT CTG TGC ATG GCT GTC CTT     1056
Thr Arg Lys Val Lys Leu Asp His Asp Tyr Leu Cys Met Ala Val Leu
            340                 345                 350

GTT CAA GAA ATA ATA AAT GCT GAT TAT GCA TTT GTC ATT CAC ACA ACC     1104
Val Gln Glu Ile Ile Asn Ala Asp Tyr Ala Phe Val Ile His Thr Thr
        355                 360                 365

AAC CCA TCT TCC GGA GAC GAC TCA GAA ATA TAT GCC GAG GTG GTC AGG     1152
Asn Pro Ser Ser Gly Asp Asp Ser Glu Ile Tyr Ala Glu Val Val Arg
    370                 375                 380
GGC CTT GGG GAA ACA CTT GTT GGA GCT TAT CCA GGA CGT GCT TTG AGT     1200
Gly Leu Gly Glu Thr Leu Val Gly Ala Tyr Pro Gly Arg Ala Leu Ser
385                 390                 395                 400

TTT ATC TGC AAG AAA AAG GAT CTC AAC TCT CCT CAA GTG TTA GGT TAC     1248
Phe Ile Cys Lys Lys Lys Asp Leu Asn Ser Pro Gln Val Leu Gly Tyr
                405                 410                 415

CCA AGC AAA CCG ATC GGC CTT TTC ATA AAA AGA TCT ATC ATC TTC CGA     1296
Pro Ser Lys Pro Ile Gly Leu Phe Ile Lys Arg Ser Ile Ile Phe Arg
            420                 425                 430

TCT GAT TCC AAT GGG GAA GAT TTG GAA GGT TAT GCC GGT GCT GGC CTC     1344
Ser Asp Ser Asn Gly Glu Asp Leu Glu Gly Tyr Ala Gly Ala Gly Leu
        435                 440                 445
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TAC GAC AGT GTA CCA ATG GAT GAG GAG GAA AAA GTT GTA ATT GAT TAC      1392
Tyr Asp Ser Val Pro Met Asp Glu Glu Glu Lys Val Val Ile Asp Tyr
    450                 455                 460

TCT TCC GAC CCA TTG ATA ACT GAT GGT AAC TTC CGC CAG ACA ATC CTG      1440
Ser Ser Asp Pro Leu Ile Thr Asp Gly Asn Phe Arg Gln Thr Ile Leu
465                 470                 475                 480

TCC AAC ATT GCT CGT GCT GGA CAT GCT ATC GAG GAG CTA TAT GGC TCT      1488
Ser Asn Ile Ala Arg Ala Gly His Ala Ile Glu Glu Leu Tyr Gly Ser
                485                 490                 495

CCT CAA GAC ATT GAG GGT GTA GTG AGG GAT GGA AAG ATT TAT GTC GTT      1536
Pro Gln Asp Ile Glu Gly Val Val Arg Asp Gly Lys Ile Tyr Val Val
            500                 505                 510

CAG ACA AGA CCA CAG ATG T GATTATATTC TCGTTGTATG TTGTTCAGAG           1585
Gln Thr Arg Pro Gln Met
        515

AAGACCACAG ATGTGATCAT ATTCTCATTG TATCAGATCT GTGACCACTT ACCTGATACC    1645

TCCCATGAAG TTACCTGTAT GATTATACGT GATCCAAAGC CATCACATCA TGTTCACCTT    1705

CAGCTATTGG AGGAGAAGTG AGAAGTAGGA ATTGCAATAT GAGGAATAAT AAGAAAAACT    1765

TTGTAAAAGC TAAATTAGCT GGGTATGATA TAGGGAGAAA TGTGTAAACA TTGTACTATA    1825

TATAGTATAT ACACACGCAT TATGTATTGC ATTATGCACT GAATAATATC GCAGCATCAA    1885

AGAAGAAATC CTTTGGGTGG TTTCAAAAAA AAA                                 1918
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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 518 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Ala Glu Trp Tyr His His Leu Leu Gln Pro Ser Ala Glu Tyr Leu Gly
  1               5                  10                  15

Ser Ile Leu Gly Val Asp Gln Trp Ala Leu Asn Ile Phe Thr Glu Glu
             20                  25                  30

Ile Ile Arg Ala Gly Ser Ala Ala Ser Leu Ser Ser Leu Leu Asn Arg
         35                  40                  45

Leu Asp Pro Val Leu Arg Lys Thr Ala Asn Leu Gly Ser Trp Gln Ile
     50                  55                  60

Ile Ser Pro Val Glu Ala Val Gly Tyr Val Val Val Val Asp Glu Leu
 65                  70                  75                  80

Leu Ser Val Gln Asn Glu Ile Tyr Glu Lys Pro Thr Ile Leu Val Ala
                 85                  90                  95
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Lys Ser Val Lys Gly Glu Glu Glu Ile Pro Asp Gly Ala Val Ala Leu
100 105 110

Ile Thr Pro Asp Met Pro Asp Val Leu Ser His Val Ser Val Arg Ala
115 120 125

Arg Asn Gly Lys Val Cys Phe Ala Thr Cys Phe Asp Pro Asn Ile Leu
130 135 140

Ala Asp Leu Gln Ala Lys Glu Gly Arg Ile Leu Leu Leu Lys Pro Thr
145 150 155 160

Pro Ser Asp Ile Ile Tyr Ser Glu Val Asn Glu Ile Glu Leu Gln Ser
165 170 175

Ser Ser Asn Leu Val Glu Ala Glu Thr Ser Ala Thr Leu Arg Leu Val
180 185 190

Lys Lys Gln Phe Gly Gly Cys Tyr Ala Ile Ser Ala Asp Glu Phe Thr
195 200 205

Ser Glu Met Val Gly Ala Lys Ser Arg Asn Ile Ala Tyr Leu Lys Gly
210 215 220

Lys Val Pro Ser Ser Val Gly Ile Pro Thr Ser Val Ala Leu Pro Phe
225 230 235 240

Gly Val Phe Glu Lys Val Leu Ser Asp Asp Ile Asn Gln Gly Val Ala
245 250 255

Lys Glu Leu Gln Ile Leu Thr Lys Lys Leu Ser Glu Gly Asp Phe Ser
260 265 270

Ala Leu Gly Glu Ile Arg Thr Thr Val Leu Asp Leu Ser Thr Pro Ala
275 280 285

Gln Leu Val Lys Glu Leu Lys Glu Lys Met Gln Gly Ser Gly Met Pro
290 295 300

Trp Pro Gly Asp Glu Gly Pro Lys Arg Trp Glu Gln Ala Trp Met Ala
305 310 315 320

Ile Lys Lys Val Trp Ala Ser Lys Trp Asn Glu Arg Ala Tyr Phe Ser
325 330 335

Thr Arg Lys Val Lys Leu Asp His Asp Tyr Leu Cys Met Ala Val Leu
340 345 350

Val Gln Glu Ile Ile Asn Ala Asp Tyr Ala Phe Val Ile His Thr Thr
355 360 365

Asn Pro Ser Ser Gly Asp Asp Ser Glu Ile Tyr Ala Glu Val Val Arg
370 375 380

Gly Leu Gly Glu Thr Leu Val Gly Ala Tyr Pro Gly Arg Ala Leu Ser
385 390 395 400

Phe Ile Cys Lys Lys Lys Asp Leu Asn Ser Pro Gln Val Leu Gly Tyr
405 410 415

Pro Ser Lys Pro Ile Gly Leu Phe Ile Lys Arg Ser Ile Ile Phe Arg
420 425 430

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Ser Asp Ser Asn Gly Glu Asp Leu Glu Gly Tyr Ala Gly Ala Gly Leu
        435                 440                 445

Tyr Asp Ser Val Pro Met Asp Glu Glu Glu Lys Val Val Ile Asp Tyr
    450                 455                 460

Ser Ser Asp Pro Leu Ile Thr Asp Gly Asn Phe Arg Gln Thr Ile Leu
465                 470                 475                 480

Ser Asn Ile Ala Arg Ala Gly His Ala Ile Glu Glu Leu Tyr Gly Ser
                485                 490                 495

Pro Gln Asp Ile Glu Gly Val Val Arg Asp Gly Lys Ile Tyr Val Val
            500                 505                 510

Gln Thr Arg Pro Gln Met
        515
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2307 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Zea mays

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:33..1943

(xi) SEQUENZBESCHREIBUNG: SEQ ID NO: 5:

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TAGTGGATCC CCCCGGGCTG CAGGGAATTC GG CAC GAG CTT GAG GGG CTA TTG      53
                                    His Glu Leu Glu Gly Leu Leu
                                     1               5

GAA GCT CGA GTT GAA CTG CGC CCT TTG CTT CTT GAT TCG CGT GAA CGC     101
Glu Ala Arg Val Glu Leu Arg Pro Leu Leu Leu Asp Ser Arg Glu Arg
         10                  15                  20

ATG AAA GAT CTT ATA TTT TTG GAC ATT GCT CTT GAT TCT ACC TTC AGG     149
Met Lys Asp Leu Ile Phe Leu Asp Ile Ala Leu Asp Ser Thr Phe Arg
     25                  30                  35

ACA GCA ATT GAA AGG TCA TAT GAG GAG CTG AAT GAT GCA GCC CCA GAG     197
Thr Ala Ile Glu Arg Ser Tyr Glu Glu Leu Asn Asp Ala Ala Pro Glu
 40                  45                  50                  55
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AAA ATA ATG TAC TTC ATC AGT CTT GTC CTT GAA AAT CTT GCG CTT TCA 245
Lys Ile Met Tyr Phe Ile Ser Leu Val Leu Glu Asn Leu Ala Leu Ser
60 65 70

ATT GAC GAC AAT GAA GAC ATC CTG TAT TGT TTA AAG GGA TGG AAC CAA 293
Ile Asp Asp Asn Glu Asp Ile Leu Tyr Cys Leu Lys Gly Trp Asn Gln
75 80 85

GCC TTG GAA ATG GCT AAG CAA AAA GAC GAC CAA TGG GCG CTC TAT GCT 341
Ala Leu Glu Met Ala Lys Gln Lys Asp Asp Gln Trp Ala Leu Tyr Ala
90 95 100

AAA GCA TTT CTT GAC AGA AAC AGA CTT GCC CTT GCG AGC AAG GGA GAA 389
Lys Ala Phe Leu Asp Arg Asn Arg Leu Ala Leu Ala Ser Lys Gly Glu
105 110 115

CAA TAC CAT AAT ATG ATG CAG CCC TCT GCT GAG TAT CTT GGC TCG TTA 437
Gln Tyr His Asn Met Met Gln Pro Ser Ala Glu Tyr Leu Gly Ser Leu
120 125 130 135

CTC AGC ATA GAC CAA TGG GCA GTC AAT ATC TTC ACA GAA GAA ATT ATA 485
Leu Ser Ile Asp Gln Trp Ala Val Asn Ile Phe Thr Glu Glu Ile Ile
140 145 150

CGC GGT GGA TCA GCT GCT ACT CTG TCT GCT CTT CTG AAC CGA TTT GAT 533
Arg Gly Gly Ser Ala Ala Thr Leu Ser Ala Leu Leu Asn Arg Phe Asp
155 160 165

CCT GTT TTA AGG AAT GTT GCT CAC CTC GGA AGT TGG CAG GTT ATA AGC 581
Pro Val Leu Arg Asn Val Ala His Leu Gly Ser Trp Gln Val Ile Ser
170 175 180

CCG GTT GAA GTA TCA GGT TAT GTG GTT GTG GTT GAT GAG TTA CTT GCT 629
Pro Val Glu Val Ser Gly Tyr Val Val Val Val Asp Glu Leu Leu Ala
185 190 195

GTC CAG AAC AAA TCT TAT GAT AAA CCA ACC ATC CTT GTG GCA AAG AGT 677
Val Gln Asn Lys Ser Tyr Asp Lys Pro Thr Ile Leu Val Ala Lys Ser
200 205 210 215

GTC AAG GGA GAG GAA GAA ATA CCA GAT GGA GTA GTT GGT GTA ATT ACA 725
Val Lys Gly Glu Glu Glu Ile Pro Asp Gly Val Val Gly Val Ile Thr
220 225 230

CCT GAT ATG CCA GAT GTT CTG TCT CAT GTG TCA GTC CGA GCA AGG AAT 773
Pro Asp Met Pro Asp Val Leu Ser His Val Ser Val Arg Ala Arg Asn
235 240 245

AGC AAG GTA CTG TTT GCG ACC TGT TTT GAC CAC ACC ACT CTA TCT GAA 821
Ser Lys Val Leu Phe Ala Thr Cys Phe Asp His Thr Thr Leu Ser Glu
250 255 260

CTT GAA GGA TAT GAT CAG AAA CTG TTT TCC TTC AAG CCT ACT TCT GCA 869
Leu Glu Gly Tyr Asp Gln Lys Leu Phe Ser Phe Lys Pro Thr Ser Ala
265 270 275

GAT ATA ACC TAT AGG GAG ATC ACA GAG AGT GAA CTT CAG CAA TCA AGT 917
Asp Ile Thr Tyr Arg Glu Ile Thr Glu Ser Glu Leu Gln Gln Ser Ser
280 285 290 295

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TCT CCA AAT GCA GAA GTT GGC CAT GCA GTA CCA TCT ATT TCA TTG GCC      965
Ser Pro Asn Ala Glu Val Gly His Ala Val Pro Ser Ile Ser Leu Ala
                300                 305                 310

AAG AAG AAA TTT CTT GGA AAA TAT GCA ATA TCA GCC GAA GAA TTC TCT     1013
Lys Lys Lys Phe Leu Gly Lys Tyr Ala Ile Ser Ala Glu Glu Phe Ser
            315                 320                 325

GAG GAA ATG GTT GGG GCC AAG TCT CGG AAT ATA GCA TAC CTC AAA GGA     1061
Glu Glu Met Val Gly Ala Lys Ser Arg Asn Ile Ala Tyr Leu Lys Gly
        330                 335                 340

AAA GTA CCT TCA TGG GTC GGT GTC CCA ACG TCA GTT GCG ATA CCA TTT     1109
Lys Val Pro Ser Trp Val Gly Val Pro Thr Ser Val Ala Ile Pro Phe
    345                 350                 355

GGC ACT TTT GAG AAG GTT TTG TCA GAT GGG CTT AAT AAG GAA GTA GCA     1157
Gly Thr Phe Glu Lys Val Leu Ser Asp Gly Leu Asn Lys Glu Val Ala
360                 365                 370                 375

CAG AGC ATA GAG AAG CTT AAG ATC AGA CTT GCC CAA GAA GAT TTT AGT     1205
Gln Ser Ile Glu Lys Leu Lys Ile Arg Leu Ala Gln Glu Asp Phe Ser
                380                 385                 390

GCT CTA GGT GAA ATA AGA AAA GTC GTC CTT AAT CTT ACT GCT CCT ATG     1253
Ala Leu Gly Glu Ile Arg Lys Val Val Leu Asn Leu Thr Ala Pro Met
            395                 400                 405

CAA TTG GTT AAT GAG CTG AAG GAG AGG ATG CTA GGC TCT GGA ATG CCC     1301
Gln Leu Val Asn Glu Leu Lys Glu Arg Met Leu Gly Ser Gly Met Pro
        410                 415                 420

TGG CCT GGT GAT GAA GGA GAC AAG CGT TGG GAG CAA GCA TGG ATG GCT     1349
Trp Pro Gly Asp Glu Gly Asp Lys Arg Trp Glu Gln Ala Trp Met Ala
    425                 430                 435

ATT AAA AAG GTT TGG GCA TCA AAA TGG AAC GAA AGA GCA TAT TTT AGC     1397
Ile Lys Lys Val Trp Ala Ser Lys Trp Asn Glu Arg Ala Tyr Phe Ser
440                 445                 450                 455

ACA CGC AAG GTG AAA CTT GAT CAT GAG TAC CTT TCG ATG GCT GTT CTC     1445
Thr Arg Lys Val Lys Leu Asp His Glu Tyr Leu Ser Met Ala Val Leu
                460                 465                 470

GTG CAA GAA GTT GTG AAT GCA GAT TAT GCT TTT GTC ATT CAT ACC ACA     1493
Val Gln Glu Val Val Asn Ala Asp Tyr Ala Phe Val Ile His Thr Thr
            475                 480                 485

AAC CCA TCG TCT GGA GAT TCT TCT GAG ATA TAT GCT GAA GTG GTG AAA     1541
Asn Pro Ser Ser Gly Asp Ser Ser Glu Ile Tyr Ala Glu Val Val Lys
        490                 495                 500

GGG CTT GGC GAG ACC CTC GTG GGA GCC TAT CCT GGT CGT GCT ATG AGC     1589
Gly Leu Gly Glu Thr Leu Val Gly Ala Tyr Pro Gly Arg Ala Met Ser
    505                 510                 515

TTT GTT TGC AAA AAA GAT GAC CTT GAC TCT CCC AAG TTA CTT GGT TAC     1637
Phe Val Cys Lys Lys Asp Asp Leu Asp Ser Pro Lys Leu Leu Gly Tyr
520                 525                 530                 535
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CCA AGC AAG CCA ATT GGT CTC TTC ATA AGG CAA TCA ATC ATC TTC CGT 1685
Pro Ser Lys Pro Ile Gly Leu Phe Ile Arg Gln Ser Ile Ile Phe Arg
540 545 550

TCC GAC TCC AAC GGT GAG GAC CTG GAA GGT TAT GCT GGA GCA GGA TTA 1733
Ser Asp Ser Asn Gly Glu Asp Leu Glu Gly Tyr Ala Gly Ala Gly Leu
555 560 565

TAT GAT AGT GTA CCG ATG GAT GAG GAG GAT GAG GTT GTA CTT GAT TAT 1781
Tyr Asp Ser Val Pro Met Asp Glu Glu Asp Glu Val Val Leu Asp Tyr
570 575 580

ACA ACT GAC CCT CTT ATA GTA GAC CGT GGA TTC CGA AGC TCA ATC CTC 1829
Thr Thr Asp Pro Leu Ile Val Asp Arg Gly Phe Arg Ser Ser Ile Leu
585 590 595

TCA AGC ATA GCA CGG GCT GGC CAT GCC ATC GAG GAG CTA TAT GGT TCT 1877
Ser Ser Ile Ala Arg Ala Gly His Ala Ile Glu Glu Leu Tyr Gly Ser
600 605 610 615

CCT CAG GAC GTC GAG GGA GTA GTG AAG GAT GGA AAA ATC TAT GTA GTC 1925
Pro Gln Asp Val Glu Gly Val Val Lys Asp Gly Lys Ile Tyr Val Val
620 625 630

CAG ACA AGA CCA CAG ATG TAGTATGTAT GCATCTATTA GACAGCTCAA 1973
Gln Thr Arg Pro Gln Met
635

TAAGCACTGT TGTACGCTTG TATGGTTGGG ACATATGGGC GTTATGGCAT GTATAGTTGT 2033

ATGCCTAGAT GTACAACACG TGTACTCGTA TATATATATA TAAATGCTGA AACAAGCATT 2093

GGTCCTGTAC TGTAGTTTCT ACATTTCATT GTCACCAATA ATTAAGTGTA CTCCTATGGC 2153

TGGGAGTCTA TGAAAATGGA CGTGTTGACT TATTGGGTAA TAAATAATTT ATATATAAAA 2213

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA CTCGAGGGGG 2273

GGCCGGTCCC AATTCGCCTA TAGTGAGTCG TATA 2307

(2) ANGABEN ZU SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 637 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

His Glu Leu Glu Gly Leu Leu Glu Ala Arg Val Glu Leu Arg Pro Leu
1 5 10 15

Leu Leu Asp Ser Arg Glu Arg Met Lys Asp Leu Ile Phe Leu Asp Ile
20 25 30

Ala Leu Asp Ser Thr Phe Arg Thr Ala Ile Glu Arg Ser Tyr Glu Glu
35 40 45

Leu Asn Asp Ala Ala Pro Glu Lys Ile Met Tyr Phe Ile Ser Leu Val
50 55 60

Leu Glu Asn Leu Ala Leu Ser Ile Asp Asp Asn Glu Asp Ile Leu Tyr
65 70 75 80

Cys Leu Lys Gly Trp Asn Gln Ala Leu Glu Met Ala Lys Gln Lys Asp
85 90 95

Asp Gln Trp Ala Leu Tyr Ala Lys Ala Phe Leu Asp Arg Asn Arg Leu
100 105 110

Ala Leu Ala Ser Lys Gly Glu Gln Tyr His Asn Met Met Gln Pro Ser
115 120 125

Ala Glu Tyr Leu Gly Ser Leu Leu Ser Ile Asp Gln Trp Ala Val Asn
130 135 140

Ile Phe Thr Glu Glu Ile Ile Arg Gly Gly Ser Ala Ala Thr Leu Ser
145 150 155 160

Ala Leu Leu Asn Arg Phe Asp Pro Val Leu Arg Asn Val Ala His Leu
165 170 175

Gly Ser Trp Gln Val Ile Ser Pro Val Glu Val Ser Gly Tyr Val Val
180 185 190

Val Val Asp Glu Leu Leu Ala Val Gln Asn Lys Ser Tyr Asp Lys Pro
195 200 205

Thr Ile Leu Val Ala Lys Ser Val Lys Gly Glu Glu Glu Ile Pro Asp
210 215 220

Gly Val Val Gly Val Ile Thr Pro Asp Met Pro Asp Val Leu Ser His
225 230 235 240

Val Ser Val Arg Ala Arg Asn Ser Lys Val Leu Phe Ala Thr Cys Phe
245 250 255

Asp His Thr Thr Leu Ser Glu Leu Glu Gly Tyr Asp Gln Lys Leu Phe
260 265 270

Ser Phe Lys Pro Thr Ser Ala Asp Ile Thr Tyr Arg Glu Ile Thr Glu
275 280 285

Ser Glu Leu Gln Gln Ser Ser Ser Pro Asn Ala Glu Val Gly His Ala
290 295 300

Val Pro Ser Ile Ser Leu Ala Lys Lys Lys Phe Leu Gly Lys Tyr Ala
305 310 315 320

Ile Ser Ala Glu Glu Phe Ser Glu Glu Met Val Gly Ala Lys Ser Arg
325 330 335

Asn Ile Ala Tyr Leu Lys Gly Lys Val Pro Ser Trp Val Gly Val Pro
340 345 350

Thr Ser Val Ala Ile Pro Phe Gly Thr Phe Glu Lys Val Leu Ser Asp
355 360 365

Gly Leu Asn Lys Glu Val Ala Gln Ser Ile Glu Lys Leu Lys Ile Arg
370 375 380

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Leu Ala Gln Glu Asp Phe Ser Ala Leu Gly Glu Ile Arg Lys Val Val
385                 390                 395                 400

Leu Asn Leu Thr Ala Pro Met Gln Leu Val Asn Glu Leu Lys Glu Arg
                405                 410                 415

Met Leu Gly Ser Gly Met Pro Trp Pro Gly Asp Glu Gly Asp Lys Arg
            420                 425                 430

Trp Glu Gln Ala Trp Met Ala Ile Lys Lys Val Trp Ala Ser Lys Trp
        435                 440                 445

Asn Glu Arg Ala Tyr Phe Ser Thr Arg Lys Val Lys Leu Asp His Glu
    450                 455                 460

Tyr Leu Ser Met Ala Val Leu Val Gln Glu Val Val Asn Ala Asp Tyr
465                 470                 475                 480

Ala Phe Val Ile His Thr Thr Asn Pro Ser Ser Gly Asp Ser Ser Glu
                485                 490                 495

Ile Tyr Ala Glu Val Val Lys Gly Leu Gly Glu Thr Leu Val Gly Ala
            500                 505                 510

Tyr Pro Gly Arg Ala Met Ser Phe Val Cys Lys Lys Asp Asp Leu Asp
        515                 520                 525

Ser Pro Lys Leu Leu Gly Tyr Pro Ser Lys Pro Ile Gly Leu Phe Ile
    530                 535                 540

Arg Gln Ser Ile Ile Phe Arg Ser Asp Ser Asn Gly Glu Asp Leu Glu
545                 550                 555                 560

Gly Tyr Ala Gly Ala Gly Leu Tyr Asp Ser Val Pro Met Asp Glu Glu
                565                 570                 575

Asp Glu Val Val Leu Asp Tyr Thr Thr Asp Pro Leu Ile Val Asp Arg
            580                 585                 590

Gly Phe Arg Ser Ser Ile Leu Ser Ser Ile Ala Arg Ala Gly His Ala
        595                 600                 605

Ile Glu Glu Leu Tyr Gly Ser Pro Gln Asp Val Glu Gly Val Val Lys
    610                 615                 620

Asp Gly Lys Ile Tyr Val Val Gln Thr Arg Pro Gln Met
625                 630                 635
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(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4329 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Zea mays

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:2..4009

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

C CCA GAT GGC ACG ACA GTG TAC AAG AAC AGG GCT CTC AGG ACA CCT 46
Pro Asp Gly Thr Thr Val Tyr Lys Asn Arg Ala Leu Arg Thr Pro
1 5 10 15

TTT GTA AAG TCA GGT GAT AAC TCC ACT CTA AGG ATT GAG ATA GAT GAT 94
Phe Val Lys Ser Gly Asp Asn Ser Thr Leu Arg Ile Glu Ile Asp Asp
20 25 30

CCT GGG GTG CAC GCC ATT GAG TTC CTC ATC TTT GAC GAG ACA CAG AAC 142
Pro Gly Val His Ala Ile Glu Phe Leu Ile Phe Asp Glu Thr Gln Asn
35 40 45

AAA TGG TTT AAA AAC AAT GGC CAG AAT TTT CAG GTT CAG TTC CAG TCG 190
Lys Trp Phe Lys Asn Asn Gly Gln Asn Phe Gln Val Gln Phe Gln Ser
50 55 60

AGC CGC CAT CAG GGT ACT GGT GCA TCT GGT GCC TCC TCT TCT GCT ACT 238
Ser Arg His Gln Gly Thr Gly Ala Ser Gly Ala Ser Ser Ser Ala Thr
65 70 75

TCT ACC TTG GTG CCA GAG GAT CTT GTG CAG ATC CAA GCT TAC CTT CGG 286
Ser Thr Leu Val Pro Glu Asp Leu Val Gln Ile Gln Ala Tyr Leu Arg
80 85 90 95

TGG GAA AGA AGG GGA AAG CAG TCA TAC ACA CCA GAG CAA GAA AAG GAG 334
Trp Glu Arg Arg Gly Lys Gln Ser Tyr Thr Pro Glu Gln Glu Lys Glu
100 105 110

GAG TAT GAA GCT GCA CGA GCT GAG TTA ATA GAG GAA GTA AAC AGA GGT 382
Glu Tyr Glu Ala Ala Arg Ala Glu Leu Ile Glu Glu Val Asn Arg Gly
115 120 125

GTT TCT TTA GAG AAG CTT CGA GCT AAA TTG ACA AAA GCA CCT GAA GCA 430
Val Ser Leu Glu Lys Leu Arg Ala Lys Leu Thr Lys Ala Pro Glu Ala
130 135 140

CCC GAG TCG GAT GAA AGT AAA TCT TCT GCA TCT CGA GTG CCC ATC GGT 478
Pro Glu Ser Asp Glu Ser Lys Ser Ser Ala Ser Arg Val Pro Ile Gly
145 150 155

AAA CTT CCA GAG GAT CTT GTA CAG GTG CAG GCT TAT ATA AGG TGG GAG 526
Lys Leu Pro Glu Asp Leu Val Gln Val Gln Ala Tyr Ile Arg Trp Glu
160 165 170 175

CAA GCG GGC AAA CCA AAC TAT CCT CCT GAG AAG CAA CTG GTA GAA TTT 574
Gln Ala Gly Lys Pro Asn Tyr Pro Pro Glu Lys Gln Leu Val Glu Phe
180 185 190

GAG GAA GCA AGG AAG GAA CTG CAG GCT GAG GTG GAC AAG GGA ATC TCT 622
Glu Glu Ala Arg Lys Glu Leu Gln Ala Glu Val Asp Lys Gly Ile Ser
195 200 205

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ATT GAT CAG TTG AGG CAG AAA ATT TTG AAA GGA AAC ATT GAG AGT AAA        670
Ile Asp Gln Leu Arg Gln Lys Ile Leu Lys Gly Asn Ile Glu Ser Lys
        210                 215                 220

GTT TCC AAG CAG CTG AAG AAC AAG AAG TAC TTC TCT GTA GAA AGG ATT        718
Val Ser Lys Gln Leu Lys Asn Lys Lys Tyr Phe Ser Val Glu Arg Ile
    225                 230                 235

CAG CGC AAA AAG AGA GAT ATC ACA CAA CTT CTC AGT AAA CAT AAG CAT        766
Gln Arg Lys Lys Arg Asp Ile Thr Gln Leu Leu Ser Lys His Lys His
240                 245                 250                 255

ACA GTT ATG GAA GAT AAA GTA GAG GTT GTA CCA AAA CAA CCA ACT GTT        814
Thr Val Met Glu Asp Lys Val Glu Val Val Pro Lys Gln Pro Thr Val
                260                 265                 270

CTT GAT CTC TTC ACC AAG TCT TTA CAT GAG AAG GAT GGC TGT GAA GTT        862
Leu Asp Leu Phe Thr Lys Ser Leu His Glu Lys Asp Gly Cys Glu Val
            275                 280                 285

CTA AGC AGA AAG CTC TTC AAG TTC GGC GAT AAA GAG ATA CTG GCA ATT        910
Leu Ser Arg Lys Leu Phe Lys Phe Gly Asp Lys Glu Ile Leu Ala Ile
        290                 295                 300

TCT ACC AAG GTT CAA AAT AAA ACA GAA GTT CAC TTG GCA ACA AAC CAT        958
Ser Thr Lys Val Gln Asn Lys Thr Glu Val His Leu Ala Thr Asn His
    305                 310                 315

ACG GAC CCA CTT ATT CTT CAC TGG TCT TTG GCA AAA AAT GCT GGA GAA       1006
Thr Asp Pro Leu Ile Leu His Trp Ser Leu Ala Lys Asn Ala Gly Glu
320                 325                 330                 335

TGG AAG GCA CCT TCT CCA AAT ATA TTG CCA TCT GGT TCC ACA TTG CTG       1054
Trp Lys Ala Pro Ser Pro Asn Ile Leu Pro Ser Gly Ser Thr Leu Leu
                340                 345                 350

GAC AAG GCG TGT GAA ACT GAA TTT ACT AAA TCT GAA TTG GAT GGT TTG       1102
Asp Lys Ala Cys Glu Thr Glu Phe Thr Lys Ser Glu Leu Asp Gly Leu
            355                 360                 365

CAT TAC CAG GTT GTT GAG ATA GAG CTT GAT GAC GGA GGA TAC AAA GGA       1150
His Tyr Gln Val Val Glu Ile Glu Leu Asp Asp Gly Gly Tyr Lys Gly
        370                 375                 380

ATG CCA TTT GTT CTT CGG TCT GGT GAA ACA TGG ATA AAA AAT AAT GGT       1198
Met Pro Phe Val Leu Arg Ser Gly Glu Thr Trp Ile Lys Asn Asn Gly
    385                 390                 395

TCT GAT TTT TTC CTA GAT TTC AGC ACC CAT GAT GTC AGA AAT ATT AAG       1246
Ser Asp Phe Phe Leu Asp Phe Ser Thr His Asp Val Arg Asn Ile Lys
400                 405                 410                 415

GCA ATT TTA AAG GAC AAT GGC GAT GCT GGT AAA GGT ACT TCT AAG GCG       1294
Ala Ile Leu Lys Asp Asn Gly Asp Ala Gly Lys Gly Thr Ser Lys Ala
                420                 425                 430

TTG CTG GAG AGA ATA GCA GAT CTG GAG GAA GAT GCC CAG CGA TCT CTT       1342
Leu Leu Glu Arg Ile Ala Asp Leu Glu Glu Asp Ala Gln Arg Ser Leu
            435                 440                 445
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ATG CAC AGA TTC AAT ATT GCA GCA GAT CTA GCT GAC CAA GCC AGA GAT      1390
Met His Arg Phe Asn Ile Ala Ala Asp Leu Ala Asp Gln Ala Arg Asp
        450                 455                 460

GCT GGA CTT TTG GGT ATT GTT GGG CTT TTT GTT TGG ATT AGA TTC ATG      1438
Ala Gly Leu Leu Gly Ile Val Gly Leu Phe Val Trp Ile Arg Phe Met
    465                 470                 475

GCT ACC AGG CAA CTA ACA TGG AAT AAG AAC TAT AAT GTG AAG CCA CGT      1486
Ala Thr Arg Gln Leu Thr Trp Asn Lys Asn Tyr Asn Val Lys Pro Arg
480                 485                 490                 495

GAG ATA AGC AAA GCA CAG GAT AGG TTT ACA GAT GAT CTT GAG AAT ATG      1534
Glu Ile Ser Lys Ala Gln Asp Arg Phe Thr Asp Asp Leu Glu Asn Met
                500                 505                 510

TAC AAA ACT TAT CCA CAG TAC AGA GAG ATA TTA AGA ATG ATA ATG GCT      1582
Tyr Lys Thr Tyr Pro Gln Tyr Arg Glu Ile Leu Arg Met Ile Met Ala
            515                 520                 525

GCT GTT GGT CGC GGA GGT GAA GGT GAT GTT GGT CAA CGC ATT CGT GAT      1630
Ala Val Gly Arg Gly Gly Glu Gly Asp Val Gly Gln Arg Ile Arg Asp
        530                 535                 540

GAG ATA TTA GTA ATA CAG AGA AAT AAT GAC TGC AAA GGT GGA ATG ATG      1678
Glu Ile Leu Val Ile Gln Arg Asn Asn Asp Cys Lys Gly Gly Met Met
    545                 550                 555

GAA GAA TGG CAC CAG AAA TTG CAC AAC AAT ACA AGC CCA GAT GAT GTA      1726
Glu Glu Trp His Gln Lys Leu His Asn Asn Thr Ser Pro Asp Asp Val
560                 565                 570                 575

GTG ATA TGC CAG GCC TTA ATT GAT TAT ATC AAG AGT GAC TTT GAT ATA      1774
Val Ile Cys Gln Ala Leu Ile Asp Tyr Ile Lys Ser Asp Phe Asp Ile
                580                 585                 590

AGC GTT TAC TGG GAC ACC TTG AAC AAA AAT GGC ATA ACC AAA GAG CGT      1822
Ser Val Tyr Trp Asp Thr Leu Asn Lys Asn Gly Ile Thr Lys Glu Arg
            595                 600                 605

CTC TTG AGC TAT GAT CGT GCT ATT CAT TCA GAA CCA AAT TTC AGA AGT      1870
Leu Leu Ser Tyr Asp Arg Ala Ile His Ser Glu Pro Asn Phe Arg Ser
        610                 615                 620

GAA CAG AAG GCG GGT TTA CTC CGT GAC CTG GGA AAT TAC ATG AGA AGC      1918
Glu Gln Lys Ala Gly Leu Leu Arg Asp Leu Gly Asn Tyr Met Arg Ser
    625                 630                 635

CTA AAG GCT GTG CAT TCT GGT GCT GAT CTT GAA TCT GCT ATA GCA AGT      1966
Leu Lys Ala Val His Ser Gly Ala Asp Leu Glu Ser Ala Ile Ala Ser
640                 645                 650                 655

TGT ATG GGA TAC AAA TCA GAG GGT GAA GGT TTC ATG GTT GGT GTT CAG      2014
Cys Met Gly Tyr Lys Ser Glu Gly Glu Gly Phe Met Val Gly Val Gln
                660                 665                 670

ATC AAT CCA GTG AAG GGT TTA CCA TCT GGA TTT CCG GAG TTG CTT GAA      2062
Ile Asn Pro Val Lys Gly Leu Pro Ser Gly Phe Pro Glu Leu Leu Glu
            675                 680                 685
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TTT GTG CTT GAA CAT GTT GAG GAT AAA TCA GCG GAA CCA CTT CCT GAG 2110
Phe Val Leu Glu His Val Glu Asp Lys Ser Ala Glu Pro Leu Pro Glu
690 695 700

GGG CTA TTG GAA GCT CGA GTT GAA CTG CGC CCT TTG CTT CTT GAT TCG 2158
Gly Leu Leu Glu Ala Arg Val Glu Leu Arg Pro Leu Leu Leu Asp Ser
705 710 715

CGT GAA CGC ATG AAA GAT CTT ATA TTT TTG GAC ATT GCT CTT GAT TCT 2206
Arg Glu Arg Met Lys Asp Leu Ile Phe Leu Asp Ile Ala Leu Asp Ser
720 725 730 735

ACC TTC AGG ACA GCA ATT GAA AGG TCA TAT GAG GAG CTG AAT GAT GCA 2254
Thr Phe Arg Thr Ala Ile Glu Arg Ser Tyr Glu Glu Leu Asn Asp Ala
740 745 750

GCC CCA GAG AAA ATA ATG TAC TTC ATC AGT CTT GTC CTT GAA AAT CTT 2302
Ala Pro Glu Lys Ile Met Tyr Phe Ile Ser Leu Val Leu Glu Asn Leu
755 760 765

GCG CTT TCA ATT GAC GAC AAT GAA GAC ATC CTG TAT TGT TTA AAG GGA 2350
Ala Leu Ser Ile Asp Asp Asn Glu Asp Ile Leu Tyr Cys Leu Lys Gly
770 775 780

TGG AAC CAA GCC TTG GAA ATG GCT AAG CAA AAA GAC GAC CAA TGG GCG 2398
Trp Asn Gln Ala Leu Glu Met Ala Lys Gln Lys Asp Asp Gln Trp Ala
785 790 795

CTC TAT GCT AAA GCA TTT CTT GAC AGA AAC AGA CTT GCC CTT GCG AGC 2446
Leu Tyr Ala Lys Ala Phe Leu Asp Arg Asn Arg Leu Ala Leu Ala Ser
800 805 810 815

AAG GGA GAA CAA TAC CAT AAT ATG ATG CAG CCC TCT GCT GAG TAT CTT 2494
Lys Gly Glu Gln Tyr His Asn Met Met Gln Pro Ser Ala Glu Tyr Leu
820 825 830

GGC TCG TTA CTC AGC ATA GAC CAA TGG GCA GTC AAT ATC TTC ACA GAA 2542
Gly Ser Leu Leu Ser Ile Asp Gln Trp Ala Val Asn Ile Phe Thr Glu
835 840 845

GAA ATT ATA CGC GGT GGA TCA GCT GCT ACT CTG TCT GCT CTT CTG AAC 2590
Glu Ile Ile Arg Gly Gly Ser Ala Ala Thr Leu Ser Ala Leu Leu Asn
850 855 860

CGA TTT GAT CCT GTT TTA AGG AAT GTT GCT CAC CTC GGA AGT TGG CAG 2638
Arg Phe Asp Pro Val Leu Arg Asn Val Ala His Leu Gly Ser Trp Gln
865 870 875

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Val Ile Ser Pro Val Glu Val Ser Gly Tyr Val Val Val Val Asp Glu
880 885 890 895

TTA CTT GCT GTC CAG AAC AAA TCT TAT GAT AAA CCA ACC ATC CTT GTG 2734
Leu Leu Ala Val Gln Asn Lys Ser Tyr Asp Lys Pro Thr Ile Leu Val
900 905 910

GCA AAG AGT GTC AAG GGA GAG GAA GAA ATA CCA GAT GGA GTA GTT GGT 2782
Ala Lys Ser Val Lys Gly Glu Glu Glu Ile Pro Asp Gly Val Val Gly
915 920 925

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Val Ile Thr Pro Asp Met Pro Asp Val Leu Ser His Val Ser Val Arg
        930                 935                 940

GCA AGG AAT AGC AAG GTA CTG TTT GCG ACC TGT TTT GAC CAC ACC ACT      2878
Ala Arg Asn Ser Lys Val Leu Phe Ala Thr Cys Phe Asp His Thr Thr
    945                 950                 955

CTA TCT GAA CTT GAA GGA TAT GAT CAG AAA CTG TTT TCC TTC AAG CCT      2926
Leu Ser Glu Leu Glu Gly Tyr Asp Gln Lys Leu Phe Ser Phe Lys Pro
960                 965                 970                 975

ACT TCT GCA GAT ATA ACC TAT AGG GAG ATC ACA GAG AGT GAA CTT CAG      2974
Thr Ser Ala Asp Ile Thr Tyr Arg Glu Ile Thr Glu Ser Glu Leu Gln
                980                 985                 990

CAA TCA AGT TCT CCA AAT GCA GAA GTT GGC CAT GCA GTA CCA TCT ATT      3022
Gln Ser Ser Ser Pro Asn Ala Glu Val Gly His Ala Val Pro Ser Ile
            995                1000                1005

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Ser Leu Ala Lys Lys Lys Phe Leu Gly Lys Tyr Ala Ile Ser Ala Glu
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CTC AAA GGA AAA GTA CCT TCA TGG GTC GGT GTC CCA ACG TCA GTT GCG      3166
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ATA CCA TTT GGC ACT TTT GAG AAG GTT TTG TCA GAT GGG CTT AAT AAG      3214
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GAT TTT AGT GCT CTA GGT GAA ATA AGA AAA GTC GTC CTT AAT CTT ACT      3310
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(A) LENGTH: 1336 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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Gly Val His Ala Ile Glu Phe Leu Ile Phe Asp Glu Thr Gln Asn Lys
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Trp Phe Lys Asn Asn Gly Gln Asn Phe Gln Val Gln Phe Gln Ser Ser
    50                  55                  60

Arg His Gln Gly Thr Gly Ala Ser Gly Ala Ser Ser Ser Ala Thr Ser
65                  70                  75                  80

Thr Leu Val Pro Glu Asp Leu Val Gln Ile Gln Ala Tyr Leu Arg Trp
                85                  90                  95

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            100                 105                 110

Tyr Glu Ala Ala Arg Ala Glu Leu Ile Glu Glu Val Asn Arg Gly Val
        115                 120                 125

Ser Leu Glu Lys Leu Arg Ala Lys Leu Thr Lys Ala Pro Glu Ala Pro
    130                 135                 140

Glu Ser Asp Glu Ser Lys Ser Ser Ala Ser Arg Val Pro Ile Gly Lys
145                 150                 155                 160

Leu Pro Glu Asp Leu Val Gln Val Gln Ala Tyr Ile Arg Trp Glu Gln
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Ala Gly Lys Pro Asn Tyr Pro Pro Glu Lys Gln Leu Val Glu Phe Glu
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        195                 200                 205

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    210                 215                 220

Ser Lys Gln Leu Lys Asn Lys Lys Tyr Phe Ser Val Glu Arg Ile Gln
225                 230                 235                 240

Arg Lys Lys Arg Asp Ile Thr Gln Leu Leu Ser Lys His Lys His Thr
                245                 250                 255

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            260                 265                 270

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        275                 280                 285

Ser Arg Lys Leu Phe Lys Phe Gly Asp Lys Glu Ile Leu Ala Ile Ser
    290                 295                 300
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530 535 540

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725 730 735

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Leu Ser Ile Asp Asp Asn Glu Asp Ile Leu Tyr Cys Leu Lys Gly Trp
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Asn Gln Ala Leu Glu Met Ala Lys Gln Lys Asp Asp Gln Trp Ala Leu
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Tyr Ala Lys Ala Phe Leu Asp Arg Asn Arg Leu Ala Leu Ala Ser Lys
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Gly Glu Gln Tyr His Asn Met Met Gln Pro Ser Ala Glu Tyr Leu Gly
820 825 830

Ser Leu Leu Ser Ile Asp Gln Trp Ala Val Asn Ile Phe Thr Glu Glu
835 840 845

Ile Ile Arg Gly Gly Ser Ala Ala Thr Leu Ser Ala Leu Leu Asn Arg
850 855 860

Phe Asp Pro Val Leu Arg Asn Val Ala His Leu Gly Ser Trp Gln Val
865 870 875 880

Ile Ser Pro Val Glu Val Ser Gly Tyr Val Val Val Val Asp Glu Leu
885 890 895

Leu Ala Val Gln Asn Lys Ser Tyr Asp Lys Pro Thr Ile Leu Val Ala
900 905 910

Lys Ser Val Lys Gly Glu Glu Glu Ile Pro Asp Gly Val Val Gly Val
915 920 925

Ile Thr Pro Asp Met Pro Asp Val Leu Ser His Val Ser Val Arg Ala
930 935 940

Arg Asn Ser Lys Val Leu Phe Ala Thr Cys Phe Asp His Thr Thr Leu
945 950 955 960

Ser Glu Leu Glu Gly Tyr Asp Gln Lys Leu Phe Ser Phe Lys Pro Thr
965 970 975

Ser Ala Asp Ile Thr Tyr Arg Glu Ile Thr Glu Ser Glu Leu Gln Gln
980 985 990

Ser Ser Ser Pro Asn Ala Glu Val Gly His Ala Val Pro Ser Ile Ser
995 1000 1005

Leu Ala Lys Lys Lys Phe Leu Gly Lys Tyr Ala Ile Ser Ala Glu Glu
1010 1015 1020

Phe Ser Glu Glu Met Val Gly Ala Lys Ser Arg Asn Ile Ala Tyr Leu
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Lys Gly Lys Val Pro Ser Trp Val Gly Val Pro Thr Ser Val Ala Ile
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Val Val Gln Thr Arg Pro Gln Met
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(2) INFORMATION FOR SEQ ID NO: 9:

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(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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(2) INFORMATION FOR SEQ ID NO: 10:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
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(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: modified_base
(B) LOCATION:15
(D) OTHER INFORMATION:/mod_base= i

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

AATGACTGCA AAGGGGGGAT GATGGA 26

Claims

1. A nucleic acid molecule encoding a protein from maize which is present in plant cells in starch granule-bound form as well as in soluble form, selected from the group consisting of:
   (a) nucleic acid molecules encoding a protein comprising the amino acid sequence indicated as in Seq ID No. 6 or in Seq ID No. 8;
   (b) nucleic acid molecules comprising the coding region of the nucleotide sequences indicated under Seq ID No. 5 or under Seq ID No. 7; and
   (c) nucleic acid molecules hybridizing to the complementary strand of a nucleic acid molecules indicated under (a) or (b); and
   (d) nucleic acid molecules the sequence of which differs from the sequence of a nucleic acid molecule of any one of (a) to (c) due to the degeneracy of the genetic code;

   as well as the respective complementary strand of such a nucleic acid molecule.

2. A vector containing a nucleic acid molecule of claim 1.

3. The vector of claim 2, wherein the nucleic acid molecule is linked to regulatory elements ensuring transcription in eukaryotic and prokaryotic cells.

4. A host cell, which is genetically modified with a nucleic acid molecule of claim 1 or with a vector of claim 2 or 3.

5. The host cell of claim 4, being a transgenic plant cell.

6. A plant containing the plant cell of claim 5.

7. A process for the production of a modified starch comprising the step of extracting from plants of claim 6 and/or from starch storing parts of such plants the starch.

8. Starch obtainable from a plant cell of claim 5, from a plant of claim 6 or by the process of claim 7.

9. A method for the production of a protein from maize in which a host cell of claim 4 is cultivated under conditions allowing for the expression of the protein and in which the protein is isolated from the cells and/or the culture medium.

10. A protein encoded by a nucleic acid molecule of claim 1 or obtainable by the method of claim 9.

11. An antibody specifically recognizing the protein of claim 10.

12. A nucleic acid molecule with a length of at least 15 nucleotides which specifically hybridizes to a nucleic acid molecule of claim 1.

13. A DNA molecule encoding an antisense-RNA complementary to the transcripts of a DNA molecule according to claim 1.

14. A DNA molecule encoding an RNA with ribozyme activity which specifically cleaves transcripts of a DNA molecule of claim 1.

15. A DNA molecule encoding an RNA which upon expression in a plant cell leads to a reduction of the expression of a

nucleic acid molecule of claim 1, due to a cosuppression effect.

16. A vector containing a DNA molecule of any one of claims 13 to 15.

17. The vector of claim 16, wherein the DNA molecule is combined with regulatory DNA elements ensuring transcription in plant cells.

18. A host cell containing a DNA molecule of any one of claims 13 to 15 or a vector of claim 16 or 17.

19. A transgenic plant cell containing a DNA molecule of any one of claims 13 to 15 in combination with regulatory DNA elements ensuring transcription in plant cells.

20. The transgenic plant cell of claim 19, in which the activity of at least one further enzyme involved in the starch biosynthesis or modification is reduced when compared to non-transformed plants.

21. The transgenic plant cell of claim 20 in which the activity of a branching enzyme is reduced.

22. The transgenic plant cell of claim 21 in which the activity of a starch granule-bound starch synthase of the isotype I (GBSS I) is reduced.

23. A transgenic plant containing a plant cell of any one of claims 19 to 22.

24. An RNA molecule obtainable by transcription of a DNA molecule of any one of claims 13 to 15.

25. A method for the production of transgenic plant cells synthesizing a modified starch characterized in that the amount of proteins of claim 11, which are synthesized in the cells in endogenous form, is reduced in the cells.

26. The method of claim 25 characterized in that the reduction of the amount of proteins of claim 11 in the cells is caused by an antisense effect.

27. The method of claim 25 characterized in that the reduction of the amount of proteins of claim 11 in the cells is caused by a ribozyme effect.

28. The method of claim 25 characterized in that the reduction of the amount of proteins of claim 11 in the cells is caused by a cosupression effect.

29. The method of any one of claims 25 to 28, wherein the enzyme activity of at least one further enzyme involved in the starch biosynthesis and/or modification is reduced.

30. The method of claim 29 wherein the enzyme is a branching enzyme.

31. The method of claim 29 wherein the enzyme is a starch granule-bound starch synthase of the isotype I (GBSSI).

32. A plant cell obtainable by a method of any one of claims 25 to 31.

33. A transgenic plant containing plant cells of claim 32.

34. A process for the production of a modified starch comprising the step of extracting from the plant of claim

23 or 33 and/or from a starch storing part of such a plant the starch.

35. Starch obtainable from plant cells of any one of claim 19 to 22 or of claim 32, of a plant of claim 23 or 33 or by the process of claim 35.

36. The starch of claim 35 characterized in that it is derived from maize.

37. Propagation material of plants of claim 6 containing plant cells of claim 5.

38. The propagation material of plants of claim 23 or 33, containing plant cells of any one of claims 19 to 22 or of claim 32.

39. The transgenic plant of claim 23 or 33 which is a maize plant.

40. Seeds of a maize plant of claim 39.

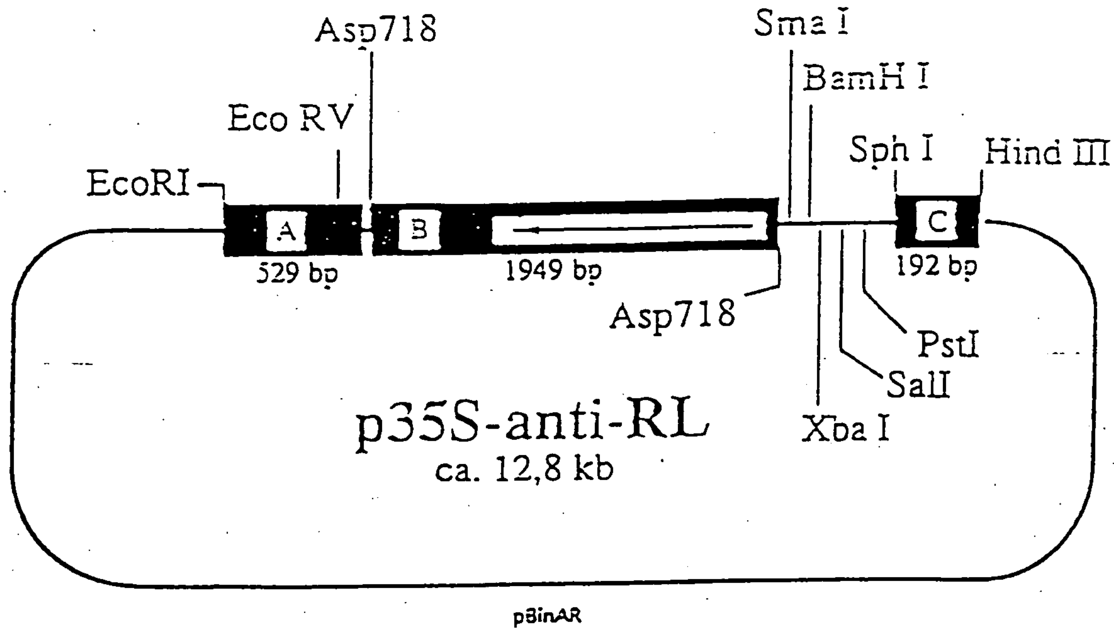
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Fig. 1

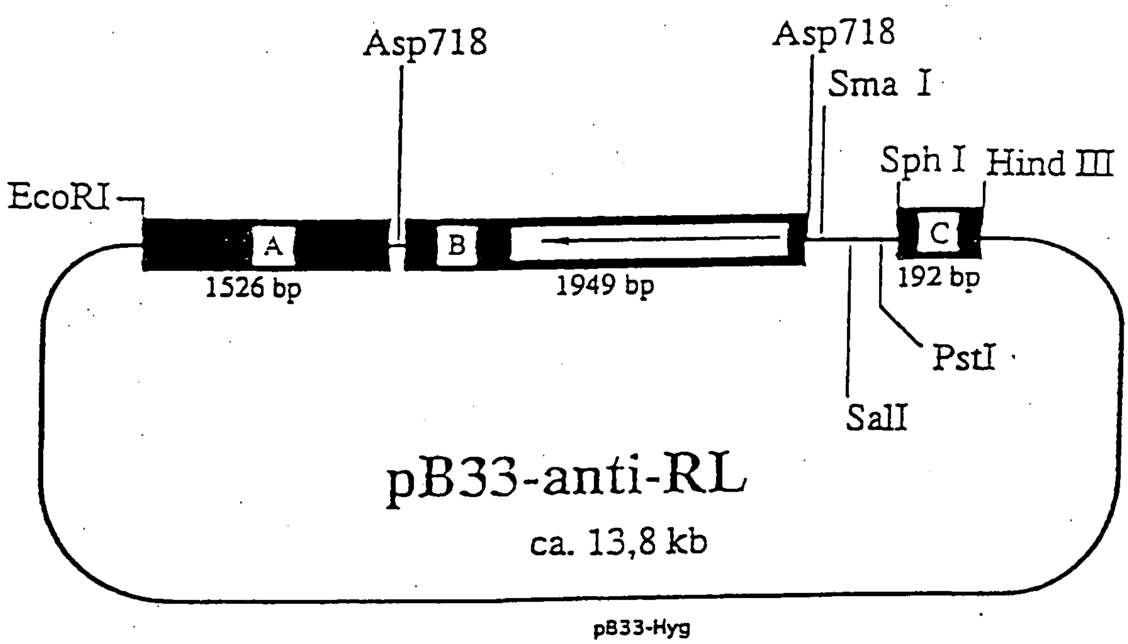
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Fig. 2

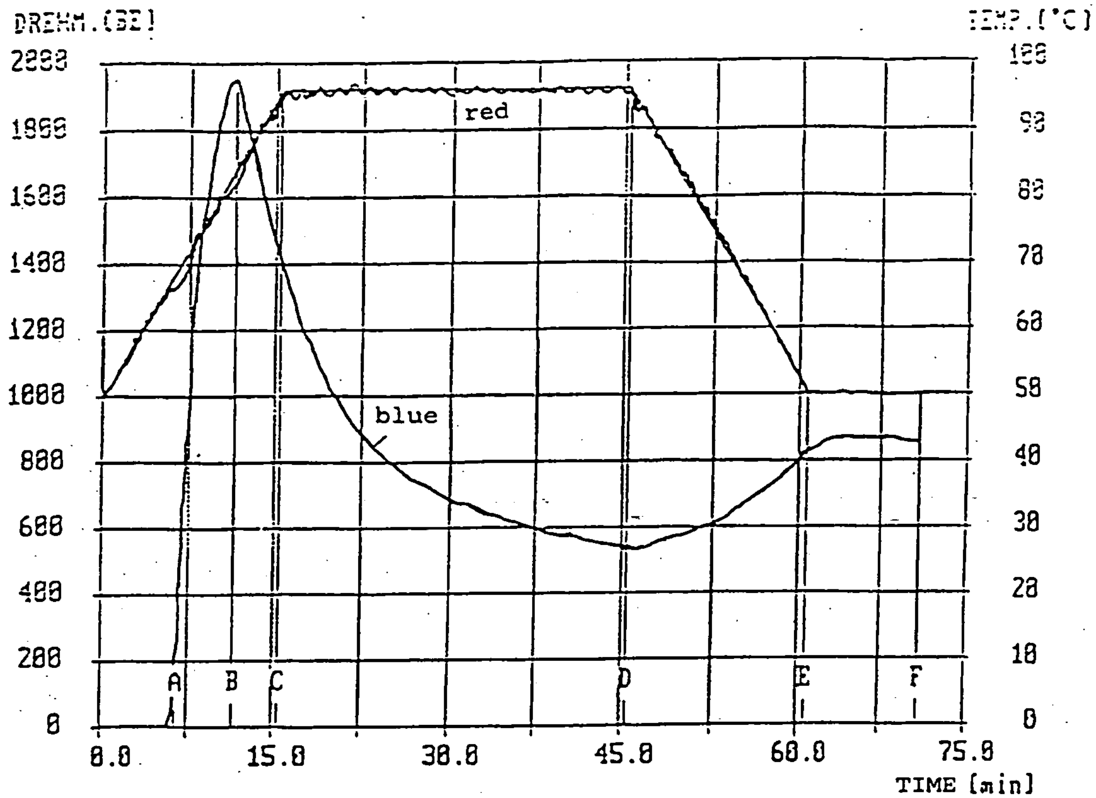
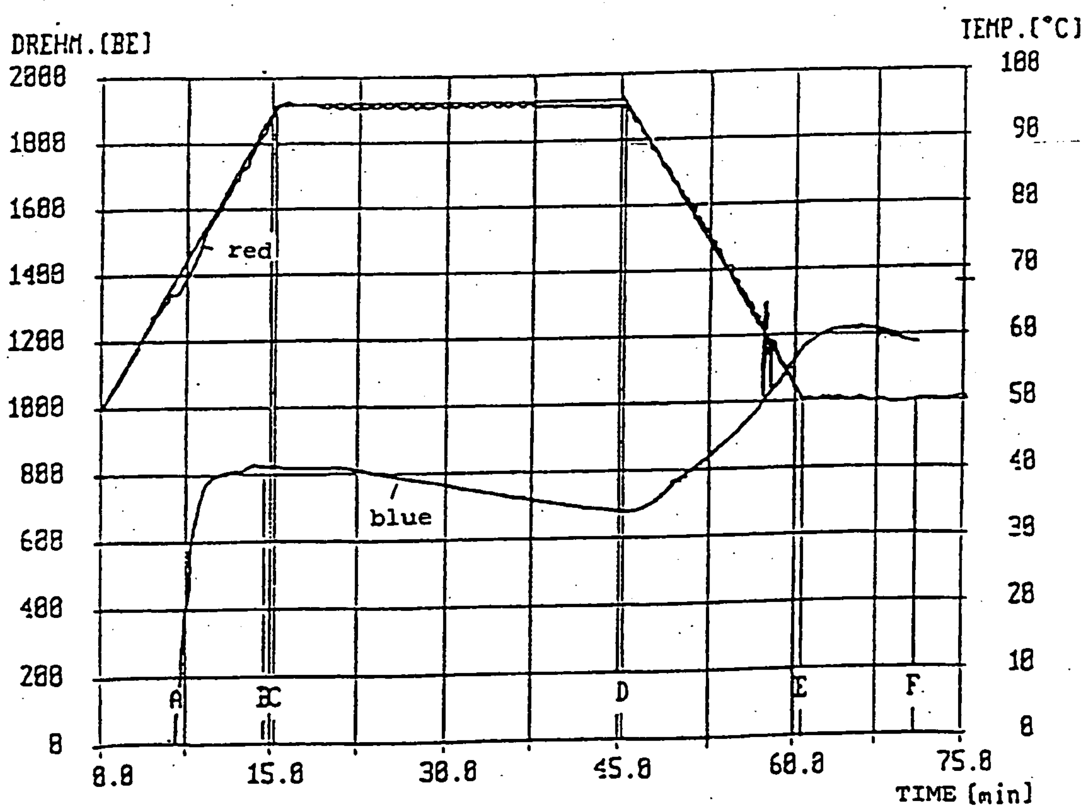


Figure 3



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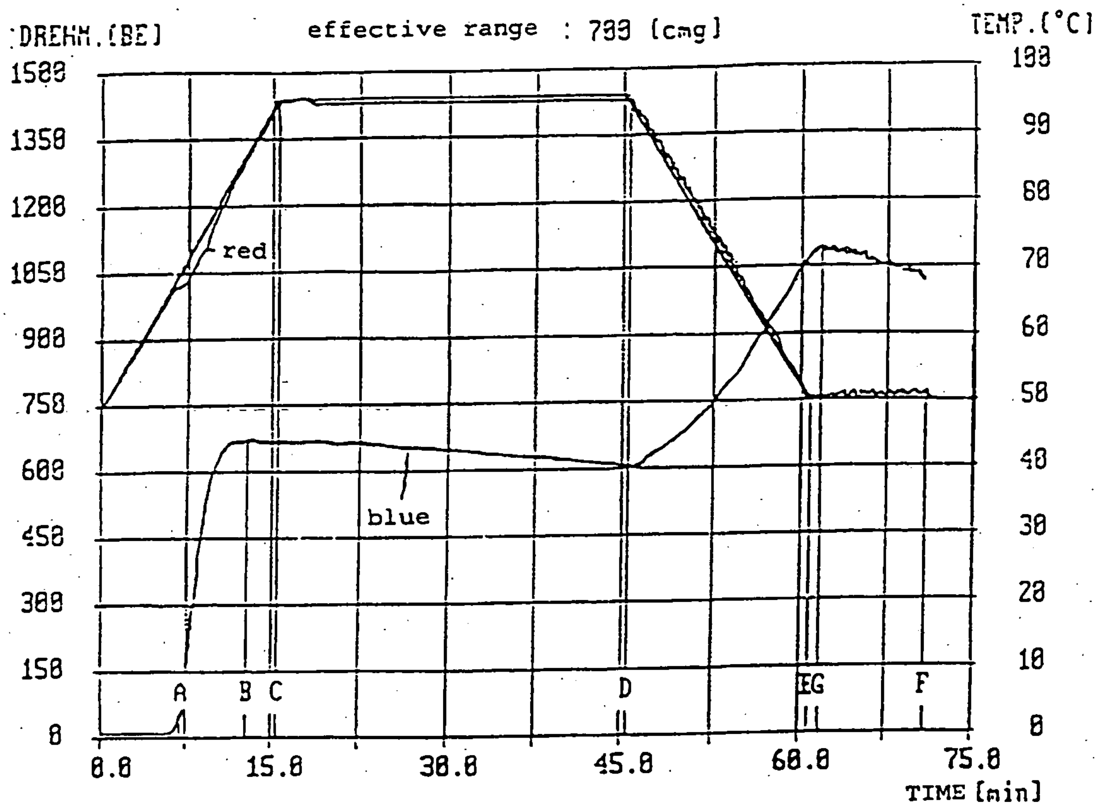
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80
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60
50
40
30
20
10
0
red
blue
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EG
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75.0
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Figure 5

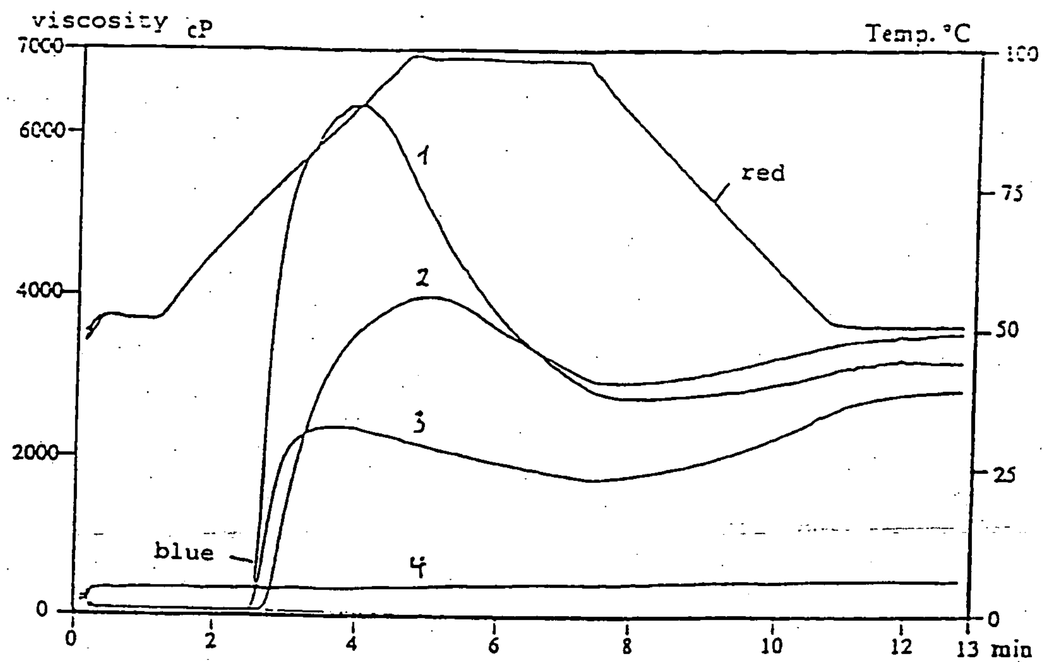


Figure 6

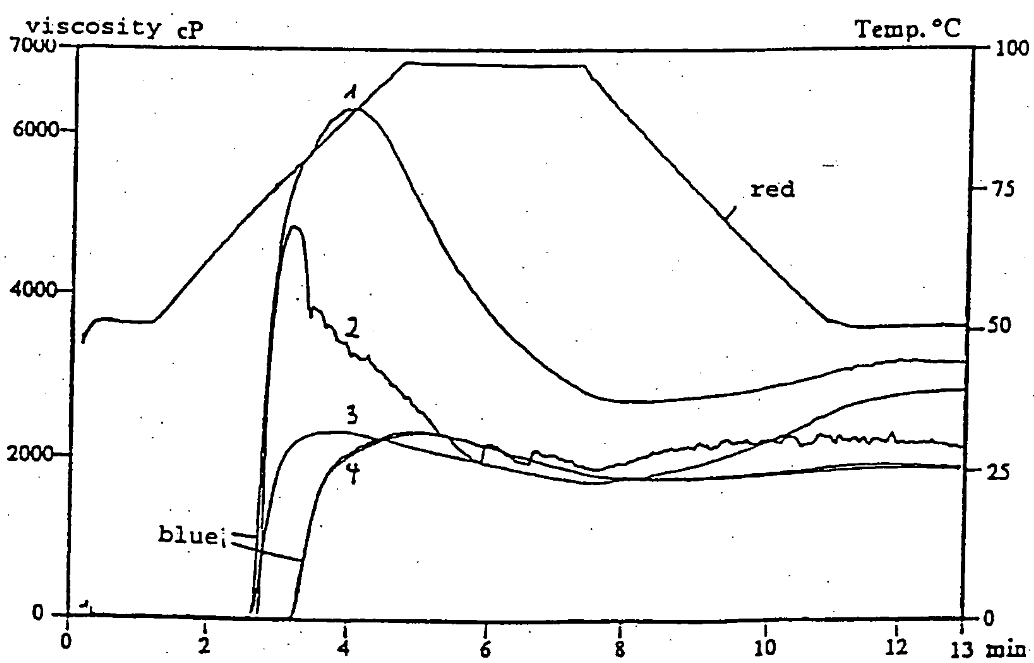
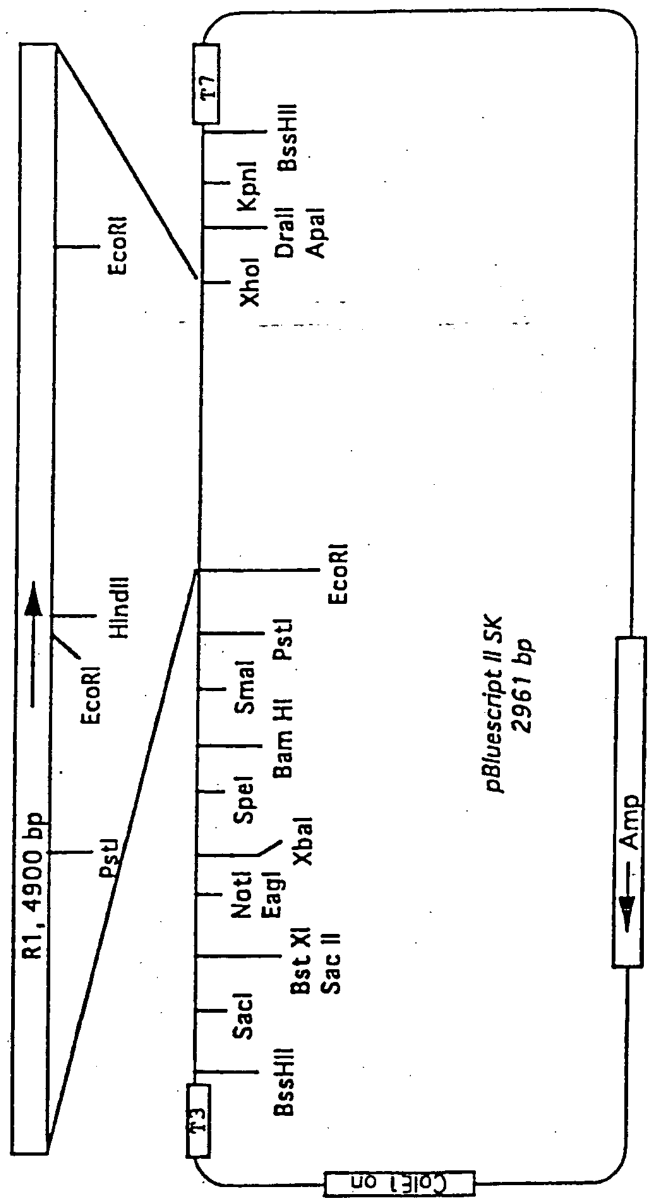


Figure 7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SpeI
XbaI
NotI
EagI
Bst XI
Sac II
SacI
BssHII
T3
ColE1 ori
pBluescript II SK
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Amp

Figure 8

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/07123

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/54 C12N15/82 C12N15/10 C12N5/10
C08B30/00 C07K14/415 C07K16/16 A01H5/00 A23L1/0522

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C08B A01H A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 94 09144 A (ZENECA LTD) 28 April 1994 see the whole document	8,35,36 1-7, 9-34, 37-40
X A	WO 95 07355 A (INST GENBIOLOGISCHE FORSCHUNG ;KOSSMANN JENS (DE); VIRGIN IVAR (DE) 16 March 1995 cited in the application see the whole document	8,35,36 20,21, 23,34, 38-40

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 April 1998	22/04/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 96 15248 A (INST GENBIOLOGISCHE FORSCHUNG ;KOSSMANN JENS (DE); SPRINGER FRANZI) 23 May 1996 see the whole document	8,35,36 20,22, 23,34, 38-40
P,X	WO 97 11188 A (KOSSMANN JENS ;LORBERTH RUTH (DE); PLANTTEC BIOTECHNOLOGIE GMBH (D) 27 March 1997 see the whole document	8,35,36

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INTERNATIONAL SEARCH REPORT

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Intern nal Application No
PCT/EP 97/07123

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